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VARIETAL DIFFERENCES IN THE RELATION BETWEEN PROTEIN CONTENT OF WHEAT AND LOAF VOLUME OF BREAD[1]

BY A. G. MCCALLA[2]

Abstract

Loaf volume obtained with the malt-phosphate-bromate formula (1 mg. of bromate per 100 gm. of flour) is highly correlated with wheat protein within any one variety of hard red spring wheat. The extremes of protein content studied were 8.0 and 20.0%, and there were few incidents of significant variation from linear correlation.

The regression of loaf volume on protein varied enormously from one variety to another. The regression coefficient is just as much an inherent varietal characteristic as is yield or protein content.

Introduction

The protein content of hard red spring wheat has for some years been considered as the best single measure of flour strength available (1, 8). This is particularly true when comparisons are confined to individual varieties of wheat (3), but high correlation coefficients have also been obtained from loaf volume and protein data involving several varieties (5, 6, 8, 12).

Not all investigators have obtained results in agreement with these conclusions (2, 7, 9, 10), but it seems likely that most of the disagreement can be explained by the baking formulas used by these investigators, since these are not suitable for high protein flours. This is well illustrated by results obtained by Larmour (8) using the basic and bromate formulas. The correlation coefficient (loaf volume—protein) with the basic formula was only 0.630 as compared with 0.903 for the same flours baked by the bromate formula. Similar results have been obtained by Harris (5, 6).

In a recent study, Aitken and Geddes (1) investigated the effect of protein content of flour on the loaf volume of bread. The distinction between this and earlier studies lies in the fact that the protein content was altered by adding prepared gluten to a low protein flour. Thus all samples were basically the same. Correlation coefficients were not given, but it is obvious that these

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were high. The authors concluded that the results yielded linear regressions over the whole range of protein content.

Waldron and Mangels (11) stressed the importance of regression coefficients as distinct from correlation coefficients in evaluating the relation between loaf volume and protein content. This is particularly important when varieties are being compared.

The studies reported in this paper were begun in an effort to explain baking results obtained with a series of wheats that included a large number of very low protein samples. It was found that, while high protein Reward wheat produced very large loaves of bread, low protein wheat of the same variety made bread of much smaller volume and poorer quality than did samples of Red Bobs and Marquis wheat of comparable protein content. This was at first attributed to the source of the material, so an intensive study of results obtained with wheats grown in comparable plots was undertaken.

Material and Methods

Material and data from a number of sources have been used in this study. Results obtained in our own laboratory are confined to those of the crops grown from 1935 to 1938 inclusive. All baking of these samples was done by one technician. The results discussed are grouped into the following series:

1. *Alberta Wheat Quality Surveys*, including results for the varieties Garnet, Marquis, Red Bobs, and Reward. This series was made up of farmers' samples, most of which were collected from machines at the time of threshing. Wheat from all parts of Alberta is included and results are for 1936, 1937, and 1938. In 1936 and 1937, each sample collected was milled and baked, and the correlation analysis was carried out with these individual results. In 1938, a protein determination was made on each sample, and then the samples were composited by variety, protein content, and official variety zones, which are based primarily on soil types. These composites were milled and baked, and the loaf volume results correlated with mean protein values.

In 1935 a preliminary survey was made, but only over a small part of the province. Composites of each variety were made up for each zone involved and the composites baked. Similar composites were made up for each variety and zone in 1936 and 1937. The combined results for the three years are included in the correlation studies of this general series.

2. *Comparative Tests at Northern Alberta Stations*, including the varieties Garnet, Marquis, Red Bobs, and Reward. Wheat was grown at six stations in 1936, seven stations in 1937, and six stations in 1938 in randomized quadruplicate six rod-row plots. Protein determinations were made on individual samples and baking tests on composites of the four replicates.

3. *Fertilizer Tests on Grey Soil*, including Garnet and Reward. Wheat was grown at Fallis in 5 × 5 Latin squares, each plot being six rod-rows in size. Five treatments with ammonium sulphate at various times and rates were used. In each of 1936 and 1937 one square was grown on wheat stubble,

and in 1938 one square on oat stubble and one on newly broken clover sod. Protein determinations were made on wheat from individual plots, and baking tests on composites of the five replicates. Results for the three years are included in the correlation analysis.

4. *Miscellaneous Tests with Reward Wheat.* Reward wheat is used in fertilizer tests carried out by the Department of Soils, University of Alberta, at Breton (grey soil) and in rotation experiments by the Experimental Substation at Beaverlodge. Milling and baking tests of the former, and protein, milling, and baking tests of the latter are made in our laboratory. The results obtained with each of these series have been subjected to a correlation analysis.

5. *Co-operative Tests with Standard and New Varieties* produced at stations over Western Canada for the Sub-committee on Plant Breeding of the Associate Committee on Plant Diseases. This series, made up of samples obtained by compositing wheat from three or four stations, contains standard varieties and all the licensed rust resistant varieties grown in Western Canada. Results for a few unnamed and unlicensed hybrids are also included. The protein results were obtained in the Grain Research Laboratory, Winnipeg, while the loaf volume results are means of those obtained at the Grain Research Laboratory and at the Universities of Saskatchewan and Alberta.

All baking results were obtained using the malt-phosphate-bromate formula (1) with 1 mg. potassium bromate per 100 gm. loaf.

Results

All statistical analyses carried out in this study follow the methods described by Goulden (4).

Studies Involving the Varieties Commonly Grown in Alberta, Series 1 to 4

A summary of the four years' results obtained with the material collected in the Alberta wheat quality survey is given in the first part of Table I. All correlation coefficients are highly significant, but in general the highest values were obtained with the series in which composites were studied. This is accounted for by the fact that peculiarities of individual samples tend to disappear when several samples are composited. This is particularly important with the survey samples, since some of these, although predominantly of the variety listed, contained other varieties as impurities. Thus a sample of Garnet carrying 10% of Red Bobs or Marquis wheat produced bread of larger volume than comparable pure Garnet.

The most important feature of these results, however, is the variability in the values of the regression coefficients. There is both a seasonal and varietal variation; the former is accounted for largely in the gradual improvement in the technique of the experimental baker. That the varietal differences are highly significant is shown by the results of an analysis of variance carried out on the regression coefficients obtained from all series in which these four varieties were included. These results are given in Table II. Based on

TABLE I

RESULTS OF UNIVERSITY OF ALBERTA TESTS ON THE VARIETIES COMMONLY GROWN IN ALBERTA

Variety	Year	No. of samples	Mean protein ($\bar{p}$), %	Mean loaf volume ($\bar{v}$), cc.	Standard deviation of p, %	Standard deviation of v, cc.	r_{vp}	b_{vp}, cc.
Series 1. Wheat quality survey								
Garnet	1936	82	10.8	522	1.50	62.4	.830	34.5
Marquis	1936	41	13.2	709	2.35	110.0	.817	38.2
Red Bobs	1936	37	12.7	719	2.11	103.1	.880	43.0
Reward	1936	30	12.8	677	2.06	131.7	.897	57.3
Garnet	1937	119	12.3	596	1.72	90.8	.884	46.8
Marquis	1937	54	13.8	806	1.89	126.9	.926	62.0
Red Bobs	1937	76	12.9	762	1.90	96.4	.897	45.5
Reward	1937	33	13.3	748	1.86	127.1	.878	59.9
Garnet	1938	8	11.9	522	1.23	72.7	.842	49.8
Marquis	1938	12	12.9	726	2.12	131.4	.964	59.9
Red Bobs	1938	14	12.4	721	1.63	94.8	.914	53.2
Reward	1938	10	14.0	772	2.09	174.6	.993	83.1
Garnet	1935–37	9	11.7	549	1.50	67.5	.956	43.2
Marquis	1935–37	11	12.9	721	1.82	95.4	.911	47.8
Red Bobs	1935–37	10	12.4	710	1.71	82.0	.987	47.4
Reward	1935–37	11	13.6	731	1.94	138.5	.970	69.4
Series 2. Comparative tests at northern Alberta stations								
Garnet	1936–38	19	13.4	654	1.83	116.4	.830	52.8
Marquis	1936–38	19	13.6	780	1.56	118.4	.898	68.4
Red Bobs	1936–38	19	12.6	745	1.94	130.0	.966	64.6
Reward	1936–38	19	15.2	906	2.15	156.4	.963	70.0
Series 3. Fertilizer tests on grey soil								
Garnet	1936–38	20	13.1	622	1.84	109.4	.961	57.1
Reward	1936–38	20	15.0	853	2.01	173.9	.955	82.7
Series 4. Miscellaneous tests with Reward wheat								
Reward (Breton)	1936–37	27	14.6	779	1.51	132.2	.892	78.1
Reward (Beaverlodge)	1936	36	13.0	714	1.35	116.8	.973	84.3
Reward (Beaverlodge)	1937	36	15.6	986	0.98	86.4	.831	73.2
Reward (Beaverlodge)	1938	36	15.1	977	0.59	77.1	.641	84.3

All correlation coefficients significant beyond the 1% point.

these results, a difference of 6.3 cc. in the regression coefficients for two varieties is significant. Thus Reward yielded a coefficient that is significantly higher than those for the other three varieties. Even this does not give a complete picture of varietal differences, however, since Garnet does not produce as large a loaf as Red Bobs and Marquis of comparable protein content. This difference can be noted by comparing the mean protein and loaf

volume values for each variety and each year. It is more clearly seen, however, in Fig. 1. Red Bobs and Marquis gave similar regression coefficients

TABLE II

ANALYSIS OF VARIANCE, REGRESSION COEFFICIENTS FOR ALL SERIES CONTAINING THE VARIETIES GARNET, MARQUIS, RED BOBS†, AND REWARD

Variance due to	D.f.	Mean square	*F*
Series	5	280.09	9.4**
Variety	3	536.11	18.0**
Error	15	29.80	
Total	23		

† *One series contained Early Triumph in place of Red Bobs.*

** *Significant beyond the 1% point.*

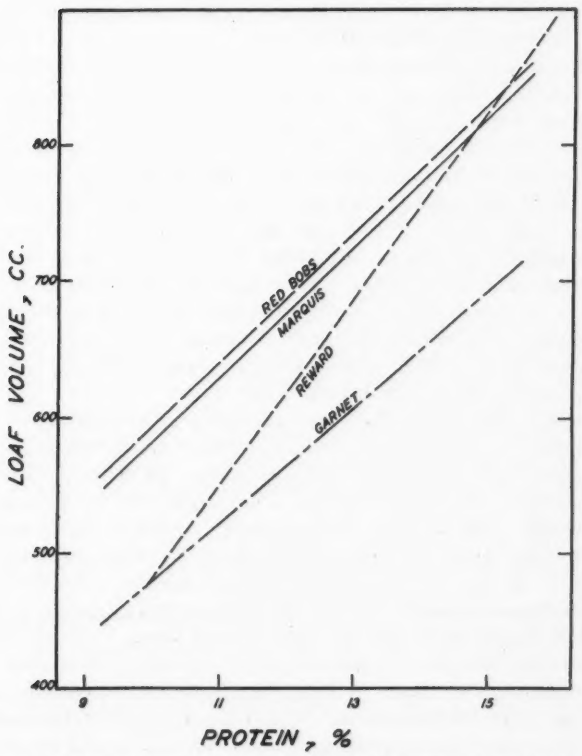

FIG. 1. *Relation between loaf volume and protein content, Series 1, 1935–37.*

and also yield bread of equal volume at any given protein level. Garnet gives about the same regression coefficient, but the regression line falls from 100 to 140 cc. below those for Red Bobs and Marquis. Reward, on the other hand, produces loaves of the same volume as Marquis and Red Bobs at 15% protein, but is little better than Garnet at 10% protein.

Most of the other results given in Table I merely confirm these conclusions. Since the material in Series 2 to 4 was grown in plots from pure seed, there is no question of contamination. In general, the correlation coefficients are somewhat higher than when large numbers of farmers' samples were studied. Particular attention is directed to the consistently high regression coefficients obtained with all series of Reward samples.

Studies with Standard and Rust Resistant Varieties, Series 5

The most comprehensive study was carried out with the co-operatively produced and tested samples of standard and rust resistant wheat varieties. This material came from points widely distributed over the three Prairie Provinces, and the loaf volume results represent the average of four replicate bakings from each of three laboratories. The results of the correlation analysis are presented in Table III.

The results with the standard varieties are in excellent agreement with those discussed in the preceding section. Despite the much greater range in protein content than in the other series, the correlation is linear, and the coefficients are very high. The regression coefficients are of the same general order as before, with no significant difference in the values for Marquis, Early Triumph (essentially Red Bobs), and Garnet, but with Reward much higher. Results for Ceres, a high quality variety, are similar to those for Marquis. The results obtained with Huron are of interest, because this variety is still grown to some extent in Alberta. It is generally considered to be a poor quality wheat, but it has frequently been found that low protein samples made relatively good bread, while high protein samples invariably were poor in quality. This variety, which gave a low regression coefficient, is apparently directly opposite to Reward in its behaviour.

There are now four high quality, licensed rust resistant varieties in Canada, and each of these has been thoroughly tested before being distributed. Three of these varieties gave regression coefficients not significantly different from those for Marquis and Early Triumph. The coefficient for Thatcher is significantly higher than that for Marquis, but the whole regression line lies above the line for Marquis. The steeper slope, therefore, does not indicate poorer quality at any protein level. Two of the rust resistant varieties, Renown and Regent, have Reward as one of the parents, but the steep regression line of Reward is not obtained with either of them. Two other Reward hybrids, however, do show evidence of this tendency, and it is of practical importance that one of these, at least, has been found deficient in quality characteristics. The third unnamed hybrid listed in Table III was discarded from the tests some years ago because it was deficient in loaf volume. That this was justified is shown by the mean protein and loaf volume results.

While it was not as poor in quality as Garnet, the regression line was essentially parallel to that of Marquis but about 70 cc. lower.

The significance of the differences in the regression coefficients for the 13 varieties listed in Table III is shown by the results of an analysis of covariance given in Table IV. These results show conclusively that there are consistent significant differences in the varieties over a period of years.

TABLE III

RESULTS ON SAMPLES GROWN AND TESTED CO-OPERATIVELY, 1933–38

Variety	Years tested	No. of samples	Mean protein ($\bar{p}$), %	Mean loaf volume ($\bar{v}$), cc.	Standard deviation of p, %	Standard deviation of v, cc.	r_{vp}	b_{vp}, cc.
Standard non-rust-resistant varieties								
Garnet	1934–38	11	15.1	797	2.28	152.8	.977	65.4
Marquis	1933–38	13	15.1	893	2.49	151.7	.951	58.0
Early Triumph	1934–37	8	14.6	900	2.43	162.8	.978	65.5
Reward	1933–38	13	16.5	999	2.15	176.9	.983	81.0
Ceres	1933–38	14	15.3	930	2.15	151.7	.950	66.9
Huron	1933–36	9	14.7	822	3.11	125.0	.935	47.8
Named, licensed rust resistant varieties								
Thatcher	1933–38	17	15.6	970	2.31	167.2	.978	71.0
Renown	1933–38	17	15.4	888	2.06	134.6	.970	63.5
Apex	1933–38	17	15.2	887	2.28	138.6	.966	58.8
Regent	1933–38	17	15.5	894	2.27	140.9	.936	58.2
Unnamed, non-licensed rust resistant varieties								
H-44-24 × Reward	1934–38	16	15.6	992	2.25	165.7	.979	72.2
H-44-24 × Reward	1934–38	16	16.3	984	1.87	142.5	.976	74.5
Hope × Reward	1933–35	8	14.2	777	2.15	136.1	.969	61.1
All above varieties	1933–38	176	15.5	926	2.25	160.5	.935	67.0

All correlation coefficients significant beyond the 1% point.

TABLE IV

TEST OF HOMOGENEITY OF VARIETAL REGRESSION COEFFICIENTS BY ANALYSIS OF RESIDUAL VARIANCE, SERIES 5

Variance due to	D.f.	Variance	F
Differences among varietal regression coefficients	12	27,349.4	18.04**
Deviations from individual varietal regressions	150	1,515.9	

** *Significant beyond the 1% point.*

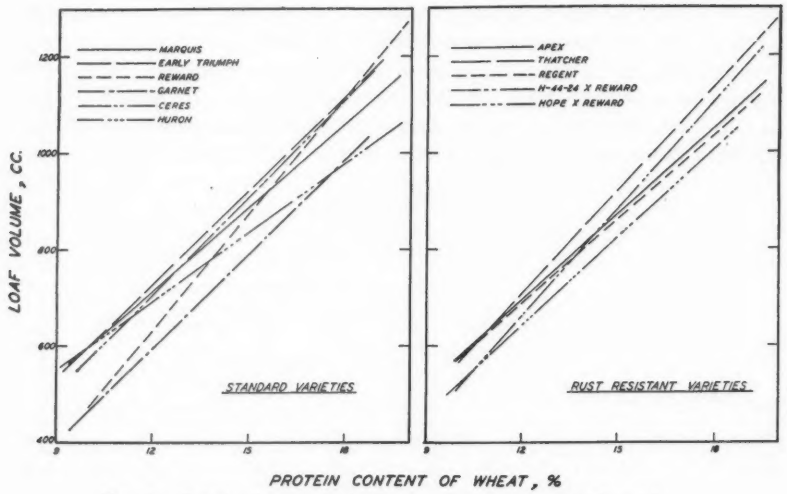

FIG. 2. *Relation between loaf volume and protein content, Series 5.*

The general relations discussed above are illustrated graphically in Fig. 2.

The values for all 13 varieties listed in Table III were included in a single correlation analysis. The results are given at the end of the table. The high degree of correlation was obtained only because there was such a wide

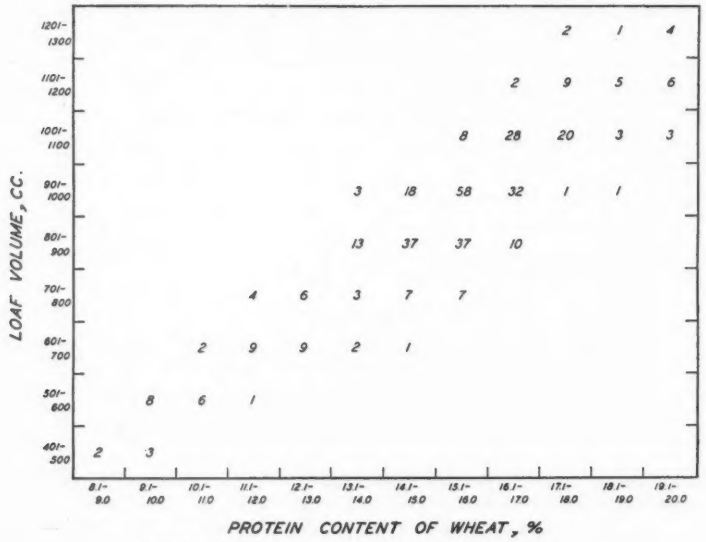

FIG. 3. *Scatter diagram for all results with Series 5.*

range in protein content. The scatter diagram (Fig. 3) made up from the results of all varieties included in these tests (this number varied from 22 to 36 in different years) shows that a range of 400 cc. was not infrequently obtained with different samples varying not more than 1% in protein. The range within individual varieties was rarely more than 150 cc.

Tests for Non-linearity of Regression

Analyses of variance have been carried out with the data for the individual varieties of Series 1, 1936 and 1937, and with the combined data for all varieties in Series 5 (Fig. 3), to test the linearity of the regressions. Only with the latter was any evidence of non-linearity found. The results of this analysis are given in Table V. The F value is significant beyond the 5% point, but

TABLE V

TEST OF LINEARITY OF REGRESSION, SERIES 5 (ALL VARIETIES)

	D.f.	Mean square	F
Deviations, means of arrays from regression line	10	1.070	2.20*
Within arrays	359	0.4872	

* *Significant to the 5% point.*

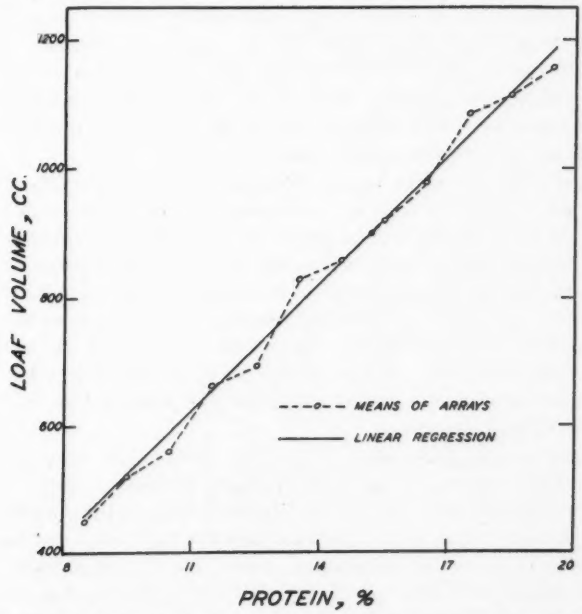

FIG. 4. *Relation between loaf volume and protein content, means of arrays, Series 5, all varieties and years; N = 371.*

when the results for the 13 samples which were above 19% in protein are omitted from the analysis, the F value becomes non-significant. The practical unimportance of the significant F value is shown by Fig. 4, in which the mean loaf volume for each array (Fig. 3) is plotted against the protein content.

It seems justifiable to conclude, therefore, that for all practical purposes the relation between protein content and loaf volume obtained with the malt-phosphate-bromate formula is linear.

Discussion

The results of this study show that the relation between protein content of wheat and loaf volume of bread is just as much a varietal characteristic as are yield and protein content. The consistently high regression coefficient obtained with Reward wheat serves to explain fully the results that originally led to this investigation. High protein Reward wheat (15% and above) makes bread of large volume, but low protein Reward is not much, if any, better than Garnet. This does not mean that Reward wheat grown under comparable conditions will not produce bread of better quality than Garnet, because under such conditions Reward will usually be about 2% higher than Garnet in protein.

From a purely local point of view, the practical importance of the present results lies in the fact that Reward has been, and is being, extensively used in breeding programs designed to give a high quality variety suitable for the northern areas in which low protein wheat is usually produced. Obviously the decidedly poor quality of low protein Reward wheat is to be regarded as an undesirable characteristic, and any new variety possessing this fault is certain to be unsatisfactory when grown on the poorer soils, no matter how good it appears to be under prairie conditions.

In contrast to Reward, the variety Red Bobs, which is generally regarded as a fairly low protein wheat, has given much better results under northern conditions than would have been expected. This is accounted for by the lower regression coefficient, and the fact that a sample of this variety containing 8% protein will make as good bread as a sample of Reward containing 10%. Such a variety as Garnet, however, is unsatisfactory, not because of a high regression coefficient, but because it requires more protein at all levels to make a loaf of specified volume. For use in the north the plant breeder must aim to produce an early wheat that is free from the undesirable characteristics of both Garnet and Reward.

The present results also indicate that, for the type of wheat produced in Western Canada, the malt-phosphate-bromate formula is very satisfactory for use in testing the baking quality of wheats in so far as the inherent strength characteristics are concerned. The fact that the large seasonal variation in correlation and regression coefficients, noted by several workers, has not been at all apparent in most of the present results, shows that this formula is much more satisfactory with wheats varying widely in protein content than are many of the formulas used by others. It seems likely that many of

the seasonal differences in regression coefficients reported by Waldron and Mangels (11) would have disappeared had such a baking formula been used by them.

Acknowledgments

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VEGETATIVE PROPAGATION OF CONIFERS

V. THE EFFECT OF INDOLYLACETIC ACID AND NUTRIENT SOLUTIONS ON THE ROOTING OF NORWAY SPRUCE CUTTINGS[1]

By N. H. Grace[2] and M. W. Thistle[3]

Abstract

Norway spruce cuttings collected in November 1938 were treated, in three experiments, with a series of solutions containing from 1/729 to 400 p.p.m. of indolylacetic acid. The effects, in all concentrations, were reduced rooting and increased mortality. The addition of nutrient salts to the treating solution further reduced rooting and increased mortality of the cuttings. These negative results of solution treatments contrast with beneficial effects already reported for application of indolylacetic acid in talc dust.

A previous communication in this series described the results of an experiment in which dormant Norway spruce cuttings were treated with indolylacetic acid in a talc carrier (6). This paper deals with experiments in which cuttings, from the same collection, were treated with a range of concentrations of indolylacetic acid in aqueous and in nutrient salt solutions. Preliminary experiments with some 5,000 solution-treated summer and winter cuttings of this species of spruce, by J. L. Farrar and the senior author, suggested that growth stimulating chemicals applied by this method had a damaging effect, at least above a concentration of 5 p.p.m. While lower concentrations did not show as much damage, definite stimulation was not noted. In consequence, the three experiments, to be described, made use of solutions ranging in concentration from 1/729 to 400 p.p.m. (parts per million).

Experimental

The material used in the three experiments was collected in mid-November 1938, the bases of the branches being placed in peat and kept outside under snow (4). Branches were from the upper part of the tree, with one exception to be mentioned subsequently. Cuttings were prepared with a heel of old wood, and ranged in length from 2 to 5 in. Each group of cuttings was representative of the different length classes. Cuttings were treated immediately after preparation. The first experiment was started on January 13, and the second and third experiments on April 7, 1939. All three experiments were carried out in the greenhouse.

Indolylacetic acid solutions were prepared by dissolving 0·1000 gm. in 1 ml. of alcohol and diluting with distilled water to give the highest concentration required. Further dilution permitted ready preparation of the lower

[1] *Manuscript received December 26, 1939.*

Contribution from the Division of Biology and Agriculture, National Research Laboratories, Ottawa. Part of a co-operative project of the Subcommittee on Forest Tree Breeding, Associate Committee on Forestry. N.R.C. No. 890.

[2] *Biochemist.*

[3] *Statistical Assistant.*

concentrations. In the first experiment there were two such solutions, one in water and one in a standard nutrient salt solution (5). Freshly prepared solutions were used for all treatments. The cuttings, four replicates of 10 in the first and second experiments and two replicates of 11 in the third, were immersed to a depth of about 3 cm. for 24 hr. in 100 cc. of solution in 400-cc. beakers.

In the first experiment there were 13 main treatments—an untreated control (planted without immersion in water or nutrient solution), a water (or nutrient) control, and a series of 11 concentrations of indolylacetic acid. These descended in geometric progression from 81 to 1/729 p.p.m., each successive concentration being reduced to one-third that of the member of the series immediately preceding. There were eight replicates of each of the foregoing 13 treatments, and the entire experiment required 1040 cuttings. Four of the replicates were treated with indolylacetic acid in water, the remaining four in solutions of the chemical in a standard nutrient solution (5). The restricted random arrangement of planting provided, in effect, four double replicates of the 12 remaining treatments, half with and half without nutrients in the treating solution. This arrangement gave two levels of precision, the first appropriate to comparisons between treatments with water and with nutrient salt solution, and the interaction of these with indolylacetic acid, and the second appropriate to comparisons between the 13 main treatments.

The second experiment comprised a series of six treatments—an untreated control, a water treated control, and treatments with 100, 200, 300, and 400 p.p.m. indolylacetic acid in water. This experiment required 240 cuttings.

The third experiment made use of cuttings from the upper and lower regions of the tree and involved three treatment groups—an untreated control, a water treated control, and a group treated with 100 p.p.m. indolylacetic acid in water. There were two replicates of 11 cuttings from each region and 132 cuttings in all. Lower cuttings were from the same tree for which results have been reported on dust treatment (4). However, upper cuttings were from a number of trees, although from the same general collection of material.

Each of the three experiments was planted in the form of simple randomized blocks.

The cuttings were planted in brown sand in propagation frames equipped with bottom heat cables. Sand temperature was maintained at approximately 72° F., while that of the room ranged around 65–70° F. for the first 12 weeks of the first experiment. Room temperature frequently rose to 80 or even 95° F. during the subsequent 12 weeks, the period in which the second and third experiments were in the bed, and prior to the final examination of the first experiment.

The cuttings were examined 12 weeks after planting and the numbers of cuttings rooted, callused, and dead were determined. Record also was made of the numbers and lengths of roots. Cuttings of the first experiment, which were either living or callused but not rooted, were replanted and observed finally at the end of a second period of 12 weeks.

Data on the number of cuttings rooted and the number dead, for Experiments 1 and 3, were subjected to analyses of variance. Only the data on the number dead were analysed for Experiment 2, as the treatments with indolylacetic acid failed to effect any rooting. For this reason, it was also impossible to make any useful statistical treatment of the data on the number and lengths of roots per rooted cutting.

Results

Experiment 1

Data for the analysis of variance and counts of the cuttings rooted and dead are given in Tables I and II. It will be observed that there are significant treatment effects with respect to both counts at both times of observation. In general, significant reduction in rooting is to be noted in practically all cases, owing to the use of indolylacetic acid. Further, all indolylacetic acid dosages produce a significant increase in mortality after both time periods. Counts of the number of cuttings rooted fail to show any interaction between nutrient and indolylacetic acid; in consequence, the results of indolylacetic acid treatment need not be given separately for water and nutrient solution treatments.

TABLE I

EXPERIMENT 1—ANALYSIS OF VARIANCE OF RESPONSE OF NORWAY SPRUCE TO NUTRIENT SALT AND PHYTOHORMONE SOLUTIONS

Source of variance	Degrees of freedom	Mean square			
		After 12 weeks		After 24 weeks	
		Number of cuttings rooted	Number of cuttings dead	Number of cuttings rooted	Number of cuttings dead
Replicates	3	.3129*	1.711***	1.460**	.5102**
Phytohormone treatments	12	.3684**	.678***	1.177**	.2262**
Error (a)	36	.0992	.158	.320	.0801
Water — nutrient differences	1	.6048	.834	9.201***	2.9156***
Interaction (W — N) × phytohormone treatments	11	.0410	.869**	.345	.0368
Error (b)	40	.2235	.306	.586	.1153

* *Exceeds mean square error, 5% level of significance.*
** *Exceeds mean square error, 1% level of significance.*
*** *Exceeds mean square error, 0.1% level of significance.*
All data transformed, $\sqrt{x + \frac{1}{2}}$ *basis (1).*

The effect of nutrient is one of damage throughout the experiment. Nutrient damage is not apparent as such at the 12-week period, except as it accentuates the increase in mortality caused by indolylacetic acid. After 24 weeks, however, injury from nutrient is strongly marked, only 13% rooting after nutrient, as against 23% after treatment with the corresponding aqueous solutions; 80% mortality with nutrient as contrasted with 61% mortality with water is also noted.

TABLE II

EXPERIMENT 1—RESPONSE OF NORWAY SPRUCE TO PHYTOHORMONE SOLUTIONS

Each figure is a mean value for 80 cuttings

Indolylacetic acid concentrations	Transformed data				Data in percentages			
	12 weeks		24 weeks		12 weeks		24 weeks	
	Cuttings rooted	Cuttings dead	Cuttings rooted	Cuttings dead	Cuttings rooted	Cuttings dead	Cuttings rooted	Cuttings dead
81 p.p.m.	.96*	2.34*	1.36*	2.88*	5	52	19	79
27 p.p.m.	.95*	1.87*	.85*	2.76*	5	36	12	74
9 p.p.m.	1.20*	1.82*	1.65	2.68*	11	32	24	68
3 p.p.m.	1.07*	1.87*	1.11*	2.81*	8	34	18	75
1 p.p.m.	.84*	1.92*	.85*	2.81*	2	34	9	75
1/3 p.p.m.	1.33	1.74*	1.66	2.63*	14	26	24	65
1/9 p.p.m.	1.12*	1.87*	1.20*	2.72*	9	34	16	70
1/27 p.p.m.	1.09*	1.64*	1.09*	2.85*	8	28	12	78
1/81 p.p.m.	1.01*	1.75*	1.19*	2.76*	6	30	20	72
1/243 p.p.m.	1.12*	1.65*	1.17*	2.64*	9	25	15	66
1/729 p.p.m.	1.04*	1.80*	1.04*	2.67*	8	30	15	68
H_2O only	1.47	1.42	1.81	2.41	20	19	34	54
Untreated	1.60	1.07	2.11	2.29	24	8	41	49
Necessary difference	.32	.40	.57	.29				

* *Differs significantly from untreated control.*

Experiment 2

The results of an analysis of variance of the data on the number of cuttings dead are presented in Table III, and it is apparent that treatment has had a highly significant effect. In Table IV are given data showing that the effect was injurious in all cases.

The untreated group had significantly fewer dead cuttings than the water control or any of the indolylacetic acid treated groups. The 100 p.p.m. treatment group had more dead cuttings than the water treated, but fewer than the groups receiving the higher concentrations, which did not differ

TABLE III

EXPERIMENT 2—ANALYSIS OF VARIANCE OF THE PROPORTION OF NORWAY SPRUCE CUTTINGS DEAD ON TREATMENT WITH PHYTOHORMONE SOLUTIONS

Source of variance	Degrees of freedom	Mean square
Blocks	3	51.28
Treatments	5	2488.52***
Error	15	69.26

Data transformed to angles (inverse sine) for analysis of variance (1).

****Exceeds mean square error, 0.1% level of significance.*

TABLE IV

EXPERIMENT 2—THE EFFECT OF PHYTOHORMONE SOLUTIONS ON THE RESPONSES OF NORWAY SPRUCE CUTTINGS

Each figure is a mean value for 40 cuttings

	Untreated	Indolylacetic acid in water, p.p.m.					Necessary difference, 5% level
		0	100	200	300	400	
No. of cuttings dead—							
Transformed* data	31.0	46.5	72.1	85.4	90.0	90.0	12.5
Per cent	28	53	88	98	100	100	
No. of cuttings rooted and callused, %	60	43	3	2	0	0	
No. of cuttings rooted, %	5	25	0	0	0	0	

* *Data transformed to angles for analysis of variance (1).*

significantly among themselves. None of the groups treated with indolylacetic acid contained any rooted cuttings, whereas appreciable rooting and callus formation occurred in the two groups without chemical treatment.

Experiment 3

Table V gives the results of analyses of variance of the number of cuttings rooted and dead, which demonstrate the existence of significant positional and treatment effects, and the means in Table VI show the nature of these effects. Cuttings from the lower part of the tree gave significantly better rooting and fewer dead cuttings than those taken from the upper region. While the untreated and water treated groups failed to differ significantly with respect to either the number of cuttings rooted or dead, both groups differed from the

TABLE V

EXPERIMENT 3—ANALYSIS OF VARIANCE OF RESPONSE OF NORWAY SPRUCE CUTTINGS FROM THE UPPER AND LOWER PART OF THE TREE TO TREATMENT WITH PHYTOHORMONE SOLUTION

Source of variance	Degrees of freedom	Mean square	
		Number of cuttings rooted†	Number of cuttings dead†
Blocks	1	654.16*	68.64
Treatments	2	723.55*	2332.14***
Position of cutting on tree	1	3240.65**	579.84*
Interaction treatment × position	2	195.59	60.31
Error	5	78.86	50.02

† *Data transformed to angles for analysis of variance (1).*
* *Exceeds mean square error, 5% level of significance.*
** *Exceeds mean square error, 1% level of significance.*
*** *Exceeds mean square error, 0.1% level of significance.*

TABLE VI

EXPERIMENT 3—RESPONSES OF NORWAY SPRUCE CUTTINGS FROM THE UPPER AND LOWER PART OF THE TREE TO TREATMENT WITH PHYTOHORMONE SOLUTION

Each figure is a mean value for 22 cuttings

	Position of cutting on tree		Necessary difference, 5% level	Untreated	Water only	Indolyl-acetic acid, 100 p.p.m.	Necessary difference, 5% level
	Upper	Lower					
Number of cuttings rooted							
Transformed* data	7.1	40.0	13.2	26.9	35.0	8.8	16.1
Per cent	4	42		27	39	5	
Number of cuttings dead							
Transformed* data	44.7	30.6	10.5	19.9	27.8	65.2	12.9
Per cent	48	32		16	23	82	

* *Data transformed to angles for analysis of variance (1).*

one receiving the 100 p.p.m. indolylacetic acid treatment. This concentration of indolylacetic acid greatly reduced rooting and increased the number of cuttings that died.

Discussion

The results of the three experiments have indicated that indolylacetic acid solutions from 1/729 to 400 p.p.m. reduced the rooting and increased mortality of Norway spruce cuttings. This finding is in agreement with that of Deuber and Farrar (2, 3). However, Thimann and Delisle have reported increased rooting of Norway spruce cuttings on solution treatment (7). It is possible that some unrecognized factor is responsible for this disagreement, since the rather marked differences between replicates noted in the propagation of conifer cuttings suggest that they are highly sensitive to apparently minor variations.

Treatment in solutions of nutrient salts for 24 hr. reduced rooting and increased mortality. Apparently this method of applying nutrient salts is too drastic, for it has been shown that small additions to the sand in which the cuttings are propagated increased rooting and decreased mortality (5).

Cuttings from the lower part of the tree gave better rooting and lower mortality than those taken from the upper region, a result in agreement with earlier findings (4). However, treatment with 100 p.p.m. indolylacetic acid solution was equally injurious to cuttings from both regions of the tree.

In Experiment 2 water treatment increased mortality over that obtained with untreated cuttings; however, water treatment also increased rooting appreciably. The explanation is found in the large number of cuttings that were merely callused. The number of cuttings that were rooted and callused was reduced by water treatment. In Experiments 1 and 3 water treatment was without significant effect.

The further increase in injury after indolylacetic acid solution treatment may be due to changes in surface tension, osmotic relations at the cell membranes, or other physico-chemical factors. In Table II it was shown that significantly more cuttings were rooted at 24 weeks following a 1/3 p.p.m. treatment than following one in which 1/729 p.p.m. of indolylacetic acid was present. The greater damage at the lower concentration suggests that absorption of toxic amounts of the chemical is not the sole cause of injury.

While solution treatment of Norway spruce cuttings with indolylacetic acid has been damaging, it must be recalled that 1000 p.p.m. in talc dust had a beneficial effect (6).

Acknowledgment

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QUALITATIVE STUDIES OF SOIL MICRO-ORGANISMS

IV. THE RHIZOSPHERE IN RELATION TO THE NUTRITIVE REQUIREMENTS OF SOIL BACTERIA[1]

By P. M. West[2] and A. G. Lochhead[3]

Abstract

Bacteria of the rhizospheres of flax and tobacco were found to possess more complex nutritive requirements than those in the corresponding control soils. The roots of even young seedlings favour the development of those types that are dependent upon a supply of thiamin, biotin, and amino nitrogen for their growth, thus suggesting that the roots may excrete significant amounts of these stimulative substances. The "rhizosphere effect" was more pronounced with susceptible than with resistant varieties of either flax or tobacco. A greater difference was found to exist between the rhizosphere and the control soil where the latter is poor than where it is richly supplied with organic matter, since liberation of growth substances by plant decomposition permits a limited development of the more typically rhizosphere forms, apart from the zone of influence of the growing plant.

Introduction

On the basis of cell morphology and taxonomic relationship, Lochhead (2) found qualitative differences between bacteria in the rhizosphere and in soil distant from plant roots. It was observed also that the growing plant appeared to be a greater factor in determining the relative predominance of different morphological types of micro-organisms in soil of definite type than did various fertilizer treatments, which, as had been shown by Taylor and Lochhead (3), exerted relatively little effect on the incidence of the various groups of bacteria. These results suggested that the plant, through the agency of root excretions, might play an important role in the nutrition of rhizosphere forms, and that in addition to differences in the relative occurrence of various morphological types in the rhizosphere compared to normal soil, there might also be significant differences in the nutritive requirements of the organisms in the two groups. This possibility was investigated, since the existence of any specific nutritive differences would not only give some indication concerning the nature of the heretofore hypothetical "plant excretions", but also explain, at least in part, the mechanism by which the plant induces the development of the characteristic rhizosphere flora.

Experimental

From the rhizospheres of Bison and Novelty flax, resistant and susceptible respectively to wilt, 100 representative bacteria were isolated according to quanti-qualitative methods previously described (2, 3). The plants were grown under greenhouse conditions in pots containing uniform soil. One hundred control organisms were isolated from the pot soil after the roots, and

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the soil adherent to them, had been removed. These organisms were inoculated into four media of increasing complexity.

(1) Base medium consisting of glucose 1.0 gm., K_2HPO_4 1.0 gm., KNO_3 0.5 gm., $MgSO_4$ 0.2 gm., $CaCl_2$ 0.1 gm., NaCl 0.1 gm., $FeCl_3$ 0.01 gm., distilled water, 1 litre. Reaction, pH 6.8.

(2) Base medium enriched with amino nitrogen: asparagine 0.5 gm., aspartic acid 0.1 gm., cysteine 0.1 gm., glycine 0.1 gm., alanine 0.1 gm. per litre.

(3) Base medium enriched with amino nitrogen, (as above) together with a combination of bacterial growth factors: thiamin (vitamin B_1) 0.1γ, biotin 0.0005γ, β-alanine 0.1γ, nicotinic acid 0.1γ, and i-inositol 0.1 mg. per ml.

(4) Base medium enriched with yeast extract 1.0 gm. per litre.

All amino acids and growth factors were used in pure form with the exception of biotin, available as a concentrate prepared by the procedure of Kögl and Tonnis (1).

The complexity of the nutritive requirements of the bacteria from rhizosphere and control soils was determined by their growth responses in the above media after five days' incubation at 28° C. The simplest requirements were shown by those organisms producing heavy growth in the base medium, and growing no more abundantly in the media to which supplements had been added. Others, while capable of development at a sub-maximal rate in the base medium, grew more rapidly on one or more of the other substrates. The most fastidious organisms were quite unable to grow in the base medium, and in these cases the presence of amino nitrogen or growth factors was absolutely essential.

Requirements of Bacteria from Flax Rhizosphere

As shown in Table I and Fig. 1, in comparison to the controls, organisms from the rhizosphere of Bison (resistant) showed an 83% proportionate increase in numbers for which amino nitrogen was either stimulative or essential, while the organisms from Novelty (susceptible) showed a 325% increase. The numbers of bacteria influenced by the combined growth factors were increased 71% in the rhizosphere of Bison, and 143% in the rhizosphere of Novelty, beyond their incidence in the control soil. These findings indicate that in addition to the morphological differences observed (2) between the predominant types in rhizosphere and control soils, there also occurs a marked difference in the nutritive requirements of the organisms in the two groups. The greater proportion of the bacteria in the flax rhizosphere appear to depend on a supply of certain amino acids or growth substances for their maximum development, while the majority of the organisms in the check soils are independent of these factors for their growth. Furthermore, it is apparent that the relative incidence of various nutritional forms differs more widely from the control soil in the rhizosphere of the susceptible flax variety than in the rhizosphere of the resistant.

TABLE I

RELATIVE INCIDENCE OF BACTERIA OF VARIOUS NUTRITIVE TYPES IN RHIZOSPHERE OF FLAX AND TOBACCO AND IN CONTROL SOILS

	Flax			Tobacco		
	Control	Rhizosphere		Control	Rhizosphere	
		Res.	Susc.		Res.	Susc.
	%	%	%	%	%	%
Growth in base medium	2.1	49.4	39.7	43.0	36.0	50.0
Amino acids essential for growth	14.7	16.9	44.1	11.8	19.5	29.0
Amino acids stimulative	0.0	10.4	19.1	6.4	13.4	22.0
Either essential or stimulative	14.7	27.3	63.2	18.2	32.9	51.0
Growth factors essential	25.6	26.4	48.2	24.7	34.0	41.8
Growth factors stimulative	4.0	24.3	23.8	12.7	14.4	19.7
Either essential or stimulative	29.6	50.7	72.0	37.4	48.4	61.5
Stimulated by yeast extract only	67.8	19.5	14.7	26.7	26.8	6.9
No stimulation in any medium	2.1	30.0	13.2	34.2	22.6	25.5
Amino acids and growth factors without effect	69.9	49.5	28.0	61.0	49.4	32.4

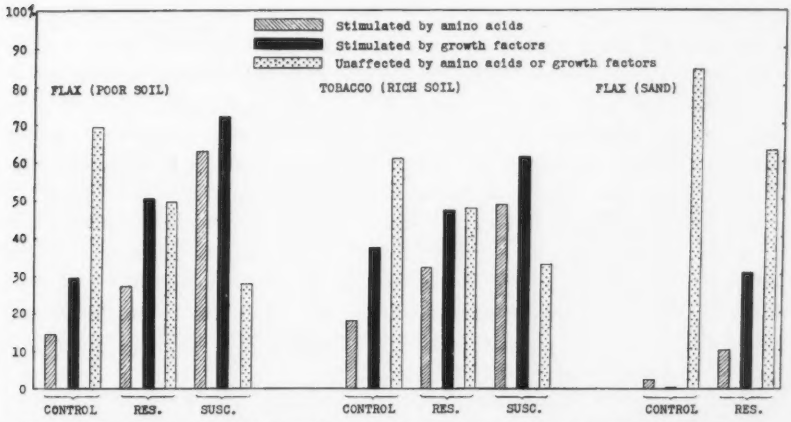

FIG. 1. *Relative incidence of rhizosphere bacteria in relation to certain nutritive requirements.*

Requirements of Bacteria from Tobacco Rhizosphere

The above experiments were repeated with bacteria from the rhizosphere of another crop, grown under field conditions. Two varieties of tobacco, one resistant and the other susceptible to black root rot, were selected for this study. As previously described, 100 isolates from the rhizospheres of resistant and susceptible tobacco varieties, together with 100 controls, were inoculated into each of the four differential media. The results of this com-

parison are also shown in Table I and Fig. 1. The rhizosphere of resistant plants showed an increase of 80% in the relative incidence of bacteria stimulated by amino acid nitrogen, while the susceptible plant rhizosphere showed an increase of 180% compared to the control soil. The number of organisms responding to the medium containing growth factors was increased 29% in the rhizosphere of resistant tobacco, and 64% in the rhizosphere of susceptible tobacco, beyond their incidence in control soil. These results are essentially in agreement with those obtained with flax, which suggests that the same fundamental factors are operative in both cases. As the tobacco soil was relatively rich, however, considerably more thiamin and biotin were already present from decomposing plant residues, thus minimizing somewhat the rhizosphere effect exerted by the plant. The poorer soil in which flax had grown therefore showed the same result in a more accentuated manner.

Relation of Control Soil to Rhizosphere Bacteria

In order to avoid entirely the interfering effect of growth substances already contained in the soil, sand was substituted in another experiment, so that the only source of growth stimulants could be the growing plant. The sand was sterilized in pots, then planted with Bison flax seedlings and inoculated with pure cultures of soil organisms, some requiring accessory growth substances, others requiring amino acids, and others growing well on the simple base medium. The initial total count, which includes approximately equal numbers of each nutritive type, was 12,800,000 per gram of sand. After three weeks in the greenhouse, representative organisms were isolated from the flax rhizosphere, together with controls from corresponding pots in which no plants had grown. The control sand contained 33,000,000 organisms per gram, but only 2% of 100 representative organisms required amino acids for their growth, while no organisms for which growth factors were essential had survived at all. On the other hand, the rhizosphere contained 1,035,000,000 organisms per gram, and, as shown in Fig. 1, approximately one-third of the total numbers was represented by bacteria for which a supply of growth factors is essential.

Thus it appears that unless the more fastidious organisms of the rhizosphere have access to certain organic substances, their development, especially among other forms which are limited by an external supply of these stimulants, is almost impossible. As has been demonstrated above, the numbers of organisms with specific growth factor requirements that occur normally in soils are therefore related in a general way to the organic matter content of the soil in question. The rich tobacco soil supported relatively high numbers, the poorer flax soil fewer numbers, and the infertile sand supported none of the bacteria having more complex nutritive requirements (Fig. 1). Even in sand, however, the predominant types of bacteria immediately adjacent to the plant roots are the more complex forms.

Essential Amino Acids

Organisms for which amino nitrogen was essential were selected for further study, in order to determine which of the ingredients of the amino acid enriched medium was specifically responsible for the observed effects. When each amino acid was tested singly no growth occurred in any case, with the possible exception of cysteine, which produced a barely detectable response. Apparently then, a combination of amino acids was necessary to yield the marked stimulative effects previously observed. On the assumption that cysteine was an essential part of such a combination, this acid was tested with each of the other ingredients of the medium. Results for two typical organisms of this group, shown in Table II, indicate that either alanine or asparagine is effective in combination with cysteine, while aspartic acid and glycine are relatively inert. By varying the concentration of cysteine in the presence of a constant amount of alanine, the optimum effect of the former was observed over the range 0.005 to 0.10 gm. per litre. Higher concentrations of cysteine than 0.01% were distinctly inhibitory. At no concentration could thioglycollic acid replace the amino acid.

TABLE II

COMBINED EFFECT OF CERTAIN AMINO ACIDS ON SELECTED ORGANISMS FROM THE RHIZOSPHERE

Additions to base medium in gm. per l.	Organism	
	XVIII-33	XVIII-21
Asparagine, 0.5	−	−
Aspartic acid, 0.1	−	−
Cysteine, 0.1	±	±
Glycine, 0.1	−	−
Alanine, 0.1	−	−
Ammonium chloride, 0.1	−	−
Cysteine, 0.1 + aspartic acid, 0.1	±	±
Cysteine + asparagine, 0.5	++++	++++
Cysteine + glycine, 0.1	++	+
Cysteine + alanine, 0.1	++++	++++
Cysteine + ammonium chloride, 0.1	+	+
Combination of all above amino acids	++++	++++

An examination of 16 pure amino acids was then made to determine whether any other sources of amino nitrogen, in combination with cysteine, were capable of providing the essential conditions for growth of this group of rhizosphere bacteria. The results of this experiment, presented in Table III, indicated that the only other amino acids able to replace alanine or asparagine were proline or hydroxyproline. the latter possessing somewhat less activity than the others. All exerted full stimulative effect as low as 0.05 gm. per litre, becoming inactive at approximately one-tenth that concentration.

The interesting question concerning whether the plant provides the rhizosphere organisms with one of these essential combinations naturally arises.

TABLE III

AMINO ACID REQUIREMENTS OF BACTERIA FROM THE RHIZOSPHERE

Additions to base medium in gm. per l.	Growth after 5 days at 28° C.		
	XVIII-33	XVIII-21	XVIII-32
Cysteine, 0.1	±	±	±
Cysteine + glycine, 0.1	+	+	++
Cysteine + alanine, 0.1	++++	++++	++++
Cysteine + serine, 0.1	±	±	±
Cysteine + valine, 0.1	±	±	±
Cysteine + leucine, 0.1	±	±	±
Cysteine + phenylalanine, 0.1	±	±	±
Cysteine + tyrosine, 0.1	±	±	±
Cysteine + tryptophane, 0.1	±	±	±
Cysteine + proline, 0.1	++++	++++	++++
Cysteine + hydroxyproline, 0.1	++++	+++	+++
Cysteine + aspartic acid, 0.1	±	±	±
Cysteine + glutamic acid, 0.1	±	±	±
Cysteine + histidine, 0.1	±	±	±
Cysteine + arginine, 0.1	±	±	±
Cysteine + lysine, 0.1	±	±	±
Cysteine + asparagine, 0.1	++++	++++	++++

In Table I it will be noted that 44% of the total numbers of organisms in the rhizosphere of Novelty flax found some such effective combination of amino acids or its equivalent, essential to their growth. The significance of these specific nutritive requirements in the light of possible plant excretions must await further study.

Essential Growth Factors

A determination of the active ingredients in the more complex medium, which contained, in addition to amino acids, a mixture of accessory growth factors, was carried out in a manner similar to the above. Each growth factor, when added to the base medium singly, was without effect. Further experiments showed that the activity of the growth factor mixture was dependent on the presence of cysteine, none of the other amino acids being of importance for this group. Using a base medium containing 0.01% cysteine, each growth substance was tested singly on the bacteria for which growth substances had been found either essential or stimulative, with the results presented in Table IV. This table includes the effect of the accessory growth factors on representative organisms from the rhizospheres of both flax and tobacco. In every case it will be noted that the active ingredient in this medium is either thiamin or biotin or both. β-Alanine, nicotinic acid, and inositol do not appear to be of significance in the nutritive needs of these bacteria. Again the possibility of root excretions suggests itself as an explanation for the relative abundance of thiamin- and biotin-requiring organisms in the rhizosphere compared to soil apart from the plant. Since the rhizosphere

TABLE IV

GROWTH FACTOR REQUIREMENTS OF 25 REPRESENTATIVE RHIZOSPHERE BACTERIA

Organism	Base medium + cysteine, 0.1 gm. per l.					
	Control	Thiamin, 0.1γ per ml.	Biotin, 0.0005γ per ml.	β-Alanine, 0.1γ per ml.	Nicotinic acid, 0.1γ per ml.	i-Inositol, 0.1 mg. per ml.
Flax						
XVI-1	+	+++	++++	++	+	+
XVI-25	−	++++	++++	±	−	−
XVI-43	−	++++	+++	+	−	−
XVI-72	−	+	++++	−	−	−
XVI-82	±	+	++++	+	+	±
XVI-91	−	++++	+++	±	±	−
XVII-5	−	++++	−	−	−	−
XIX-1	−	++++	++++	−	−	−
XVIII-2	−	++	++++	−	−	−
XVI-8	±	±	++++	±	±	±
XVI-13	++	+++	++++	++	++	++
XVI-32	++	+++	++++	++	++	++
XVI-36	+	+++	++++	+	+	+
Tobacco						
C62	−	−	++++	−	−	−
C68	−	−	++++	−	−	−
C89	−	++	++++	−	−	−
C 5	−	−	++++	−	−	−
C19	−	+++	++++	−	−	−
R11	−	−	++++	−	−	−
R82	−	−	++++	−	−	−
S66	−	−	++++	−	−	−
S22	−	++++	++++	−	−	−
S77	−	++	++++	−	−	−
B16	−	++++	−	−	−	−
B 9	−	−	++++	−	−	−

effect is exerted by the plant even in the seedling stage, it appears that the liberation of growth substances into the rhizosphere occurs more probably by excretion than by decomposition. More recent research has yielded direct evidence in support of this view (4).

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NITRIFICATION UNDER AND AFTER ALFALFA, BROME, TIMOTHY, AND WESTERN RYE GRASS

III. COMPOSITION OF HAY CROP RESIDUES[1]

By R. Newton[2] and R. S. Young[3]

Abstract

Proximate analyses of roots (to plow depth) and stubble in one-, three-, and five-year-old sods, considered in relation to sequence effects as judged by the nitrogen absorption of the first two wheat crops after each age of sod, indicated the nitrogen content of the hay crop residues to be the dominant influence. Alfalfa was much superior to the grasses, a result apparently of the higher quantity of nitrogen returned to the soil and of the narrower C : N ratio in its residues. Timothy led the grasses, contributing the highest quantity of nitrogen in residues with the lowest percentage of crude fibre and the narrowest ratio of crude fibre to nitrogen-free extract. Brome contributed more residual nitrogen than western rye, but was slightly inferior in sequence effects.

In the first paper of this series (2) experiments at Edmonton, Alberta, extending over a period of eight years were reported, in which the nitrogen absorption of alfalfa (*Medicago sativa* L.), brome (*Bromus inermis* Leyss.), timothy (*Phleum pratense* L.), and western rye grass (*Agropyron tenerum* Vasey), and of succeeding wheat crops, was used as an indication of the rate of soil nitrification. The stubble and roots (to six-inch depth) of these crops were included in the estimations of yield and nitrogen absorption. It will now be appropriate to examine in greater detail the quantity and composition of the hay crop residues, in relation to the rates of nitrification found after breaking one-, three-, and five-year-old sods.

Analytical Data

The roots and stubble were subjected to proximate analyses by standard methods. Though the roots had been carefully washed before drying and grinding for analysis, the apparent ash content of the grass roots was so high as to lead to further examination of some of the original material. Under a low-power microscope it could be seen that fine particles of sand were so firmly embedded in the surface of the roots as to be impracticable of washing out. The analytical results are therefore expressed in Table I on the basis of organic matter as found by combustion. The table is defective with respect to western rye grass, five-year-old sod being unavailable because

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TABLE I

COMPOSITION OF ORGANIC MATTER IN ROOTS AND STUBBLE

Sod	Age of sod, yr.	Crude protein, %		Ether extract, %		Crude fibre, %		N-free extract, %	
		Roots	Stubble	Roots	Stubble	Roots	Stubble	Roots	Stubble
Alfalfa	1	13.0	17.0	3.54	.98	26.3	41.0	57.2	41.0
	3	13.6	11.0	1.09	.75	28.1	46.6	57.2	41.6
	5	15.1	15.1	1.28	.74	29.4	43.2	54.3	40.9
Brome	1	7.2	8.9	.43	1.42	35.8	39.3	56.6	50.3
	3	7.6	4.0	.18	.60	31.2	37.2	61.0	58.2
	5	7.0	4.3	.67	—	32.6	—	59.7	—
Timothy	1	6.4	6.2	.94	1.91	31.9	25.6	60.7	66.3
	3	10.3	5.8	.57	1.18	30.8	28.2	58.3	64.9
	5	7.1	3.3	.65	.70	26.1	28.0	66.2	68.0
Western rye	1	9.2	6.5	1.60	2.19	35.6	41.2	53.6	50.1
	3	11.1	6.4	.49	.63	33.5	38.3	54.9	54.7

killed by "take-all"; also with respect to brome grass stubble from five-year-old sod, some of the analyses being omitted because the abnormally low yield of stubble (see Table II) provided insufficient material in the sod samples taken before plowing.

The alfalfa residues were obviously much richer in protein than the grass residues. The grass stubble also shows a greater tendency to become leaner in the older sods, possibly owing to the inclusion of leached residues from earlier hay cuttings. Western rye roots were higher in protein than those of the other grasses in the two cases in which comparison is possible; in one of these cases its stubble was also richer; but the variability of the results makes caution necessary in their interpretation.

The mixed fatty substances represented by the ether extract occurred in variable but generally small concentration. While it is known that fats decompose slowly in the soil and it has been suggested that infertility may result from their accumulation (3, p. 463–5), it seems safe to assume that any differential effect of these hay crop residues arising from such a cause during the comparatively short period of this experiment would be negligible. Ether extract will therefore be dismissed from further consideration in this paper.

Crude fibre was generally lower in the alfalfa than in the grass roots, while in the stubble the situation was reversed. Among the grass roots there were no striking differences in this respect, though timothy was lowest, especially in the five-year-old sod. In the stubble, timothy had definitely less crude fibre than the others.

In Table II the quantities of organic matter and nitrogen returned to the soil by the residues are compared with the nitrogen absorbed by two succeeding wheat crops. In one-year-old grass sods the stubble exceeded the

TABLE II

CONSTITUENTS RETURNED TO SOIL IN SODS, IN RELATION TO NITROGEN ABSORBED BY TWO SUCCEEDING WHEAT CROPS

Sod	Age of sod, yr.	Organic matter, lb./acre			Nitrogen, lb./acre		
		Roots	Stubble	Total	In residues	In first wheat crop	In first + second wheat crops
Alfalfa	1	3847	2134	5981	138	67	120
	3	7304	657	7961	171	104	144
	5	6737	637	7374	178	66	132
	Mean	5963	1143	7105	162(154)*	79(85)*	132(132)*
Brome	1	2036	2497	4533	59	54	107
	3	6564	408	6972	82	65	104
	5	4488	199	4687	52	52	103
	Mean	4363	1035	5397	64(70)*	57(60)*	105(105)*
Timothy	1	2573	2814	5387	54	48	101
	3	5695	811	6506	102	85	134
	5	6538	595	7133	77	58	119
	Mean	4935	1407	6342	78(78)*	64(66)*	118(118)*
Western rye	1	901	2901	3802	43	56	107
	3	3179	1263	4442	69	66	108
	Mean	2040	2082	4122	56	61	108

* *Means of one- and three-year-old sods in parentheses.*

roots in quantity, strikingly so in western rye grass. In three- and five-year-old grass sods the relation was strongly reversed. Alfalfa roots exceeded the stubble at all three ages. If the entire grass root system had been measured, rather than only the part contained to six-inch depth, the brome and timothy roots also would undoubtedly have exceeded the corresponding stubbles at all ages*. Therefore in considering the significance of these residues, more weight must be attached to the root analyses than to the stubble analyses.

In general, the weight of organic matter in the residues reached a maximum in three-year-old sods, though timothy continued to make some later gain. Alfalfa excelled the grasses in weight of organic residues, a superiority that is much enhanced in terms of nitrogen, by reason of the higher percentage of protein in the alfalfa residues. Only alfalfa returned to the soil much more than enough nitrogen for the immediately succeeding wheat crop. Actually alfalfa returned more than enough for two succeeding wheat crops.

The ratios of carbohydrate to protein and of crude fibre to nitrogen-free extract, given in Table III, must be considered as expressions of factors

* *Professor T. K. Pavlychenko, University of Saskatchewan, Saskatoon, in a personal communication states that he has usually found in grass sods about two-thirds of the total root material in the top six inches.*

TABLE III

RATIO CARBOHYDRATE TO PROTEIN AND CRUDE FIBRE TO NITROGEN-FREE EXTRACT IN HAY CROP RESIDUES

Sod	Age of sod, yr.	Ratio carbohydrate* to protein			Ratio crude fibre to nitrogen-free extract		
		Roots	Stubble	Total	Roots	Stubble	Total
Alfalfa	1	6.4	4.8	5.7	.46	1.00	.61
	3	6.3	8.0	6.4	.49	1.12	.53
	5	5.6	5.6	5.6	.54	1.06	.57
Brome	1	12.9	10.0	11.1	.63	.78	.71
	3	12.1	24.0	12.5	.51	.64	.52
	5	13.2	22.0†	13.4	.55	—	—
Timothy	1	14.4	14.8	14.6	.53	.39	.45
	3	8.6	16.2	9.2	.53	.43	.52
	5	13.1	29.0	13.7	.39	.41	.40
Western rye	1	9.7	14.1	12.7	.66	.82	.78
	3	8.0	14.5	9.2	.61	.70	.64

* *Carbohydrate taken as sum of crude fibre and nitrogen-free extract.*

† *To make this calculation, the missing value for ether extract in Table I was taken as 0.70.*

modifying the rate and manner of decomposition of the residues. Protein-rich residues should decompose rapidly, with little temporary immobilization of soil nitrates by assimilation in the bodies of micro-organisms engaged in the destruction of carbonaceous material. Crude fibre is a resistant fraction, while nitrogen-free extract contains the readily fermentable, energy-yielding substances. Narrow ratios for both of these factors should therefore indicate favourable conditions for nitrification.

Table III shows that the ratio of carbohydrate to protein is much narrower in alfalfa than in the grasses. Among the grasses, any differences in this respect are obscured by the variations in the results. The roots of brome and timothy seem much alike, while those of western rye, as far as can be judged by the one- and three-year-old sod samples, have a narrower ratio. It also appears that in sods more than one year old the grass roots have a much narrower ratio than the stubble. The grass stubble shows a progressively wider ratio with increasing age, a corollary of the behaviour in regard to protein content noted earlier.

With respect to the ratio of crude fibre to nitrogen-free extract, the only consistent difference between alfalfa and the grasses is the higher value in alfalfa stubble. Generally speaking, this is offset by a narrower ratio in the alfalfa roots. Among the grasses, timothy is generally lowest, especially in stubble and combined residues, in which it has the lowest ratios of all the crops. Western rye, on the other hand, is generally highest.

Discussion

Obviously nitrification in the soil used in these experiments was by no means wholly dependent for its substrate on the residues of the immediately preceding hay crops. The fertile, black Edmonton soil in question is high in organic matter and nitrogen. Analyses recorded in the second paper of this series (2, Table XXV) give the following mean values of 83 determinations of total nitrogen percentage in each of the main soil levels during the course of the investigation: surface, 0.60; subsurface, 0.28; subsoil, 0.10. On the basis of a specific gravity of 1.1 for surface soil, 1.4 for subsurface, and 1.5 for subsoil, there would be nearly 9,000 lb. of nitrogen per acre in the top 6 in. and over 28,000 lb. in the top 40 in. of soil. A seasonal nitrification of 50 to 100 lb. per acre, as measured by absorption into the wheat plants, seems negligible by comparison. But the main bulk of organic matter in the soil consists of an accumulation, over a long period of time, of the more resistant fractions of plant residues, together with the products of microbial growth on these residues. Waksman (4, p. 127) believes humus nitrogen to be held in lignin-protein complexes, decomposing only slowly. Fresh residues may thus have an influence on immediate fertility quite disproportionate to their relative quantity.

In the first paper of this series (1) it was shown that the mean nitrogen absorption per acre of wheat plants following one-, three-, and five-year-old sods for six, four, and two successive years, was: after alfalfa, 63.4 lb.; after timothy, 58.6 lb.; after western rye, 56.3 lb.; after brome, 51.9 lb. The second paper (2) showed that when the sods were plowed up, nitrates accumulated in the alfalfa plots to a greater extent than in the grass plots, and to a lesser extent generally in the brome plots than in the timothy and western rye plots. In the present paper comparisons of after effects of the hay plant residues are restricted to the first two years following each age of sod. On this basis the pre-eminence of alfalfa is definitely confirmed by Table II, and the grasses on the average fall in the order just indicated, though with substantial deviations in individual cases.

The dominant influence of nitrogen content of residues is clearly shown by a comparison of alfalfa with the grasses, though the relative importance of absolute quantity and of ratio to other constituents is not so easily discernible, since alfalfa not only excels in quantity of nitrogen but has also the narrowest ratio of carbohydrate to protein. Among the grasses, timothy residues in three-year-old sod possessed the highest quantity of nitrogen, also a comparatively low C : N ratio, and were followed by the highest wheat-nitrogen absorption.

Pound for pound, the nitrogen of western rye residues seemed more completely assimilated by succeeding wheat crops than that of any other of these hay crops, not excepting alfalfa. This may signify merely that because western rye residual nitrogen was smallest in quantity, succeeding crops drew more heavily on other organic nitrogen reserves in the soil. The fact that

alfalfa, with the highest quantity of residual nitrogen, had the lowest percentage of this absorbed by the first wheat crop, and that timothy occupies a corresponding position among the grasses, lends colour to this view.

To compare the sequence effects of all three grasses in Table II, one must look only at the wheat-nitrogen absorption following one- and three-year-old sods. Brome was on the average slightly inferior to western rye and definitely inferior to timothy. This order does not quite correspond with that of the relative quantities of nitrogen supplied by their residues, which was: timothy, brome, western rye. Brome had a wider C : N ratio in its roots than western rye, though a slightly narrower ratio of crude fibre to nitrogen-free extract in both roots and stubble. One case in which the nitrogen absorption of the first wheat crop is distinctly less than would be expected from the return by the grass residues is that after three-year-old brome. In this case the brome residues had a decidedly wider C : N ratio than those of the other grasses. This is also the case in which it was pointed out in Part II (2, Fig. 2) that there was a phenomenal number of fungi (mainly *Penicillia*) in the soil. Possibly some constituent of brome residues, or its closely knit sod as already suggested (1), modifies the microbial balance in such a way as to reduce the supply of nitrates to the growing crop. Looking for further explanations of the position of timothy, we find its residues had the lowest percentage crude fibre and the narrowest ratio of crude fibre to nitrogen-free extract.

As pointed out in the first paper (1), the effects of timothy on succeeding crops did not turn out in this case in accordance with expectation. They are however in harmony with the facts deduced in the present paper. Both percentage and absolute quantity of nitrogen, and the percentage of crude fibre and ratio of this to readily fermentable carbohydrates in the residues of hay crops, appear to affect the availability of the nitrogen to succeeding crops.

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NITRIFICATION UNDER AND AFTER ALFALFA, BROME, TIMOTHY, AND WESTERN RYE GRASS

IV. OXIDATION-REDUCTION POTENTIALS AND CARBON DIOXIDE PRODUCTION[1]

By J. G. Malloch[2] and R. S. Young[3]

Abstract

Changes in the redox potentials of the soils and crop residues were caused mainly by the action of micro-organisms. No relation between the potential measurements and the yield or composition of the crops in the field could be demonstrated. All four forage plants have some fraction that was responsible for a marked drift in potential, but which disappeared early in the process of decomposition.

The greatest production of carbon dioxide occurred in soils under the hay crops, and it decreased with lapse of time after breaking. The production was greatest under and after alfalfa followed by brome. When the composition of the crop residues is taken into account, a relation between the carbon dioxide production and nitrate production can be demonstrated. The carbon dioxide production of decomposing residues decreases rapidly from a high initial value, giving further evidence of the presence of a readily decomposable constituent.

Introduction

In the first paper of this series (4) a comprehensive investigation of the sequence effects of alfalfa and certain grasses was outlined. Major emphasis has been placed on the effect of these crops on the rate of nitrification, but attention was also given to the general rate of decomposition, not only because it is important in itself but because there is evidence that nitrification is affected by the C:N ratio and by the decomposition of the non-nitrogenous constituents of the plants. Oxidation processes play a large part in this decomposition, and it was decided to make a special investigation of this phase of the problem. Both the intensity and the capacity factors received attention, the former by oxidation-reduction potential measurements and the latter by determination of carbon dioxide production.

Crop residues consisting of combined roots and stubble collected in the course of the main experiment, and samples of the top six inches of soil, were the experimental materials. The residues used came from four-year-old plots of alfalfa, brome, timothy, and western rye grass; the soils came also from the foregoing and from plots that had grown wheat for one year and three years following respectively three years and one year of these forage crops.

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Oxidation-reduction Potentials

The measurement of these potentials was undertaken to determine if there were any characteristic differences between the four forage crops used in the experiment and, if so, whether they were related to the field results. In the absence of any knowledge of the systems involved it could not be expected that any strict interpretation could be placed on the data. The potentials are simply relative measurements of an unspecified characteristic of the samples.

Method

The potentials were measured by means of a Leeds and Northrup Type K potentiometer and bright platinum electrodes of wire and foil. These were cleaned frequently by immersion in hot aqua regia. The potentials were found to be reproducible to within 0.1 mv.

The samples to be measured were placed in 100-cc. test tubes and stoppered. They were thoroughly shaken to incorporate the air or nitrogen before the first measurement of each series, but for subsequent measurements the electrodes were inserted with as little disturbance as possible.

Dried Roots and Stubble

Preliminary experiments had shown differences between the redox behaviour of fresh timothy residues and those of other crops. The initial experiments were therefore made with this kind of material. Water extracts were made from the residues and these were divided into two portions, one of which was buffered to pH 6.25, approximately the acidity of Edmonton loam on which the material was grown. Measurements of Eh were made at two-hour intervals up to 14 hr. and then at 24 hr. Only the initial and final Eh readings and any intermediate ones necessary to define the rate of change are given.

The readings for buffered and unbuffered extracts did not show any great differences and consequently only the results with buffered extracts are presented. The first experiment was repeated in the presence of nitrogen instead of in the presence of air. The gas was obtained from a commercial cylinder and, while the usual precautions to remove oxygen were taken, there probably were traces of oxygen present.

The next experiment was designed to study the effect of sterilization and subsequent inoculation with a suspension of soil micro-organisms. The sterilization was accomplished by use of a Pasteur-Chamberland filter.

The results of these experiments are shown together in Table I.

The untreated extracts show a marked change in potential in the presence of air. This change is very much reduced when the extracts are sterilized, although the initial level remains almost identical. On the addition of a suspension of soil micro-organisms the measurements were similar to those on untreated extracts, but the shift from positive to negative Eh took place several hours later, possibly owing to the induction period of the organisms.

TABLE I

EFFECT OF BACTERIAL ACTION ON THE REDOX POTENTIAL OF WATER EXTRACTS OF GROUND ROOTS AND STUBBLE

Treatment	Time, hr.	Eh, volts			
		Alfalfa	Brome	Timothy	Western rye
In presence of air					
Untreated	0	.55	.45	.48	.50
	8	.49	.38	.38	.39
	10	−.31	−.23	−.10	−.31
	24	−.33	−.33	−.32	−.33
Sterilized	0	.58	.46	.47	.50
	14	.55	.44	.44	.45
	24	.16	.15	.14	.20
Inoculated	0	.56	.46	.48	.51
	8	.47	.38	.38	.44
	10	.08	.25	.25	.29
	14	.04	.15	.20	.14
	24	−.33	−.32	−.29	−.32
In presence of nitrogen					
Untreated	0	.35	.35	.38	.35
	12	.35	.22	.25	.24
	14	.05	.09	.13	.15
	24	.03	.07	.10	.13
Sterilized	0	.35	.35	.35	.35
	24	.32	.32	.33	.33
Inoculated	0	.38	.38	.39	.38
	14	.32	.32	.29	.29
	24	.22	.24	.23	.21

It is clear that bacterial action is responsible for most of the change in potential, although there is a drift even in the sterile extracts. The immediate cause of the changes in potential was the disappearance of the dissolved oxygen. Shaking of the suspensions caused a return to the initial value. However, the changes can still be regarded as indicative of bacterial action since this is responsible for using up the oxygen supply.

The changes in the presence of nitrogen are much less marked and they begin from a lower initial level. The sterile extracts were quite stable, but as there was a slight drift when organisms were present, it seems that some bacterial action proceeded even when nitrogen was used. It is possible, however, that traces of oxygen were present. The measurements in the presence of air seem to be more informative and the use of nitrogen was discontinued.

The differences between the grasses are slight, particularly in the inoculated series, where the introduced flora was identical for all three. There is no evidence of differences that could be related to any effect the growing of these

grasses might have on subsequent crops. Alfalfa gives a higher initial value than the grasses and the change in potential takes place more rapidly. Bacterial action must have proceeded at a greater rate, and since this holds where a common inoculum was used it must be attributed to a difference in composition between the grasses and alfalfa.

Fermenting Roots and Stubble

The potentials of the crop material in process of decomposition are of more practical interest than those of fresh residues. The various crop residues were mixed with sand, brought to a moisture content equal to 50% of the water-holding capacity and allowed to undergo decomposition at 25° C. for varying periods of time. The potentials were measured in buffered water extracts. As with the fresh residues, the effect of adding suspensions of organisms to the water extracts after sterilization was tried. The results are given in Table II.

TABLE II

Effect of fermentation on the redox potentials of water extracts of mixtures of sand and crop residues

Sample	Time, hr.	Eh, volts			
		Alfalfa	Brome	Timothy	Western rye
After mixing					
Untreated	0	.49	.43	.41	.45
	8	.25	.15	.26	.30
	10	.08	−.33	−.13	.08
	12	−.06	−.33	−.18	−.01
	24	−.32	−.33	−.33	−.33
Sterilized and inoculated	0	.51	.46	.49	.54
	8	.11	.25	.30	.28
	10	−.33	−.05	.02	.05
	12	−.33	−.12	−.07	−.09
	24	−.33	−.34	−.33	−.33
After 3 weeks' fermentation					
Untreated	0	.52	.50	.50	.52
	14	.40	.42	.32	.41
	24	.28	.24	.20	.28
Sterilized and inoculated	0	.52	.53	.50	.50
	10	.22	.17	.09	.23
	14	.05	.00	.02	.10
	24	−.16	−.18	−.17	−.15
After 6 weeks' fermentation					
Untreated	0	.58	.59	.59	.58
	24	.53	.52	.51	.53
Sterilized and inoculated	0	.56	.57	.54	.55
	8	.50	.50	.48	.51
	24	.45	.44	.42	.49

In the series run directly after the preparation of the sand-residue mixtures there are marked changes in potentials in both parts of the experiment, and in this respect the results are similar to those obtained with residues alone. However, in this case there are differences between the grass residues, the change in brome being most rapid. As decomposition progresses the change in potential decreases until at six weeks the potentials are relatively stable over the 24-hr. period and all the crops are alike.

The samples were all shaken at the end of the 24-hr. measurement period to incorporate oxygen. The potentials of the extracts of freshly mixed sand and residues returned to the initial values, but the three- and six-weeks series underwent no change. With the freshly mixed samples bacterial action is rapid and the oxygen supply is a limiting factor. This does not hold for the series that had undergone partial decomposition. The difference can be explained by postulating the presence of a readily decomposable fraction in the fresh residues which disappears quite early in the process of decomposition. The values obtained after six weeks' fermentation might be expected to have the most practical significance, but in this case no differences exist between the crops.

Soils

To get more direct information on the potentials existing when the residues were decomposing under field conditions, soil samples were taken from plots growing their third crop of wheat following one year's growth of alfalfa and the grasses, from plots growing the first crop of wheat following three years' growth of the forage crops, and from the plots growing the four forage crops. The potential measurements were made on buffered soil suspensions in the presence of air. The data are given in Table III.

TABLE III

Redox potentials of soil suspensions

Soil	Time, hr.	Eh, volts			
		Alfalfa	Brome	Timothy	Western rye
1 year forage, 3 years wheat	0	.55	.56	.53	.54
	24	.51	.51	.50	.51
3 years forage, 1 year wheat	0	.53	.54	.54	.53
	24	.52	.52	.51	.51
4 years forage	0	.59	.55	.56	.54
	24	.45	.46	.47	.48

There are no essential differences between the soils growing wheat. Neither the previous crop nor the length of time it grew affect the potentials. The soils under the forage crops show a slight drift in potential and this can be attributed to the presence of the same transitory substance that caused the drift in the experiments with residues.

Some experiments were done in which residues were added to the soil and allowed to decompose. The results with extracts made after various periods of fermentation were as expected from previous experiments. There was a drift in potential in extracts made during the initial stages of decomposition, with slight differences in the rate between the different crops, but by the eleventh day the potentials for all samples became stable at 0.57 to 0.60 v.

The investigation failed to disclose any differences in the characteristics of the four forage crops that could be related to the sequence effects of these crops in the year in which the experiments were performed, and which are discussed in the first paper of the series (4).

Carbon Dioxide Production

Method

A review of the many methods for the determination of carbon dioxide production in soil described in the literature shows that the majority of investigators have concluded that aspiration over the soil gives a truer measure of microbiological activity than aspiration through the soil, the latter method producing an abnormal soil atmosphere. The former procedure was therefore adopted.

The apparatus and the method of determining the carbonate were essentially those used by Newton and Anderson (2). The blank for the entire determination, which includes errors arising from impurities in chemicals, leakages in the apparatus and any inefficiency in the towers used to wash the entering air free from carbon dioxide, was small and quite constant. In 11 determinations the value of the blank varied only from 2.28 mg. to 2.54 mg. of carbon dioxide and the appropriate corrections were made in the apparent carbon dioxide production of the experimental samples. The titration was checked by use of a solution containing sodium carbonate equivalent to 20.76 mg. of carbon dioxide. The values obtained in five trials varied from 20.64 mg. to 20.70 mg. with an average of 20.68 mg.

In the experiments 100-gm. portions of dry soil were weighed into a 300 cc. Erlenmeyer flask. When plant residues were added an amount corresponding to one gram of organic matter was incorporated. The soil was then brought to a moisture content equal to 50% of the water-holding capacity and the flasks were placed in an incubator at 25° C. The moisture content of the samples was maintained by addition of distilled water when necessary.

Three techniques for surface aspiration of the samples were tried: (i) the flasks were attached to the absorption towers continuously and aspirated for 20 min. at the end of a 24-hr. period; (ii) the flasks were attached continuously and were aspirated for 20 min. at 6, 12, and 24 hr.; (iii) the flasks were attached to the towers only for 20 min. aspiration at the end of a 24-hr. incubation. In parallel determinations the amounts of carbon dioxide found using the three techniques, were 81.2 mg., 85.2 mg. and 79.5 mg. respectively. The

last method gave somewhat lower results but the difference is not great and it was adopted because of its obvious convenience.

Soils

The plots were sampled by taking two six-inch borings from each of the four replicates and compositing. Duplicate determinations were made on these composites. The carbon dioxide produced during the first 24 hr. was determined and thereafter during the 24-hr. period following 3, 6, 9, and 12 days' incubation. The results reported in Table IV give the averages of the five determinations.

TABLE IV

Carbon dioxide production of soils growing wheat and forage crops

Crop	CO_2 produced in 24 hr. at 25° C., mg.			
	Alfalfa	Brome	Timothy	Western rye
Wheat, 3rd year after 1 yr. forage	3.8	3.0	3.1	3.1
Wheat, 1st year after 3 yr. forage	4.6	4.2	3.9	3.6
Forage, 4th yr.	6.4	8.1	4.9	4.7

The rate of decomposition, as indicated by the carbon dioxide production, is higher in the newly broken sods than it is in the plots that were broken two years previously. It is higher still in the plots growing forage crops. This is no doubt connected with the observation that the amount of crop residues did not accumulate as the sods grew older (4, p. 219). The explanation that in older sods "decomposition apparently proceeded at a greater rate than accretion by new growth" receives support from the high rate of carbon dioxide production.

Reference to the previous papers of this series shows that these results bear no direct relation to the yields obtained in the field (4) nor to the number of micro-organisms present at the time of sampling (1). As carbon dioxide production is a measure of decomposition, and nitrogen is presumably released from the residues at a rate proportional to this and to its relative abundance in the residues, the relation between carbon dioxide production and nitrate formation was investigated. Since it can be assumed that, in plots growing wheat for the first year following the plowing of three-year-old sods, the composition of the material decomposing in the soil is essentially the same as that of the residues from the previous crop, the data from these plots (3) can be used to test this relation. Root material far exceeds stubble in these residues and the composition of the former was taken as representative of the decomposing material. The calculated relative nitrate production was obtained by dividing the carbon dioxide production rate by the C : N ratio (3). This can be compared with the actual relative amounts produced in the soils, obtained by adding the amount of nitrogen absorbed by the wheat crop (4) to the amount remaining in the soil at harvest (1). No correction is necessary for the amount previously present, as nitrates were present only as a trace at

the time of plowing the forage sods. The two sets of figures were brought to a common basis by totalling each of them and expressing the individual results as percentages of the respective totals.

TABLE V

COMPARISON OF CALCULATED AND ACTUAL RELATIVE NITRATE PRODUCTION RATES

Crop	Ratio of carbonaceous to nitrogenous constituents	CO_2 production, mg.	Relative nitrate production rate	
			Calculated	Actual
Wheat after alfalfa	6.3	4.6	37	31
Wheat after brome	12.1	4.2	18	17
Wheat after timothy	8.6	3.9	23	26
Wheat after Western rye	8.0	3.6	23	26

The agreement between the relative rates of nitrate production actually found in the field and those calculated from the carbon dioxide production is surprisingly good, and, while the data are not extensive enough to constitute conclusive proof, a relation between carbon dioxide production and nitrate production is definitely indicated. The alfalfa gives the highest value in both calculated and observed nitrate production, and brome the lowest, with the other two grasses similar and intermediate. The carbon dioxide production was higher in soil after brome than after the other grasses but, because of the lower percentage of nitrogen present in the residues, more carbonaceous material had to be decomposed and the nitrate production was lower.

DECOMPOSING FORAGE CROP RESIDUES

A comparison of the susceptibility of the different residues to decomposition was attempted by adding these to portions of the same soil. The mixtures were then incubated at 25° C. for 35 days and the carbon dioxide production determined at intervals. In the results reported in Table VI the carbon dioxide production of the soil alone has been deducted and the figures given are for the gas produced from the residues only.

TABLE VI

PRODUCTION OF CARBON DIOXIDE FROM DECOMPOSING CROP RESIDUES AFTER SUCCESSIVE PERIODS OF INCUBATION AT 25° C.

Period of incubation, days	CO_2 produced in 24 hr., mg., from 1 gm. organic matter			
	Alfalfa	Brome	Timothy	Western rye
1	70.5	50.8	53.5	53.8
4	46.0	38.3	32.7	43.9
7	22.5	20.6	15.1	21.4
11	19.2	18.3	19.8	19.1
14	13.1	12.5	13.1	14.1
18	5.2	3.8	7.4	9.0
21	3.7	4.3	5.5	9.1
25	3.8	4.6	5.1	4.6
28	1.9	2.4	4.5	4.3
32	1.9	2.3	3.4	2.6
35	1.8	2.2	3.2	2.2

The initial rate is very high and the values do not drop for about four weeks to those found in the previous experiment with soil from the plots. During this period the readily decomposable fraction of the residues, to which we earlier attributed the drift in oxidation-reduction potentials, was presumably used up. The time necessary for the measurements to become relatively stable, indicating the disappearance of this fraction, is of the same order in both experiments.

At the end of the experimental period timothy was decomposing faster than the other crops, alfalfa being the slowest. There is no evidence that the depressive effect of timothy on subsequent crops, which is sometimes observed, is due to difficulty in decomposing the residues.

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CANADIAN WILTSHIRE BACON

I. OUTLINE OF INVESTIGATION AND METHODS[1]

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Abstract

This paper introduces an investigation involving bacterial, chemical, and physical measurements on bacon and pickle from 22 packing plants. The objects were to determine the over-all variation occurring in practice, the general sources of the variation, and the specific effect of certain curing practices. Methods employed for shipping the samples, sampling, and analyses are described.

It was found that small samples of curing pickle change slightly in nitrite and bacterial content during shipping periods approaching a week's duration, but that these changes are of negligible importance from a practical standpoint if the pickles are kept at temperatures near the freezing point.

Introduction

During recent years the export of bacon as Wiltshire sides* from Canada to Great Britain has assumed considerable importance in Canadian agricultural economy. As a result, investigation of the many problems associated with the manufacture and export of this type of bacon has become increasingly necessary. The present paper, introductory to a series forthcoming from these laboratories, outlines the scope of an investigation into a number of these problems, and describes the methods used for transporting the experimental sides and pickle from the packing plants to the laboratory, and the sampling and analytical methods employed.

The packing plants engaged in the export bacon trade in Canada are widely distributed and the period required for transporting the product naturally varies considerably. A survey of the methods used for making Wiltshire sides showed that, although the individual plants adhered quite closely to their own particular method, there was considerable variability in the practices

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* *For a general description of Wiltshire sides, method of curing, and typical compositions of bacon and pickle, the reader is referred to the publications of the Food Investigation Board, London, England (1–4).*

adopted in different plants. This survey, however, gave little indication of the extent to which these variations affected the composition and quality of the bacon. Consequently, the present investigation was undertaken to determine: the over-all variation of the bacon with respect to the several measurements; the main general sources of variation; and more specific information on the effect of certain curing practices.

The over-all variation of Canadian bacon with respect to an individual property may be divided into: differences between sides from the same plant; differences between sides from different plants; and differences due to age from curing. The differences between sides from the same plant represent the combined effect of inherent differences in the curing quality of different carcasses and slight variations in the treatment each carcass may receive from the time of slaughter to the baling of the finished product. The difference between sides from different plants includes the sources of variation recognized above within factories, and also the additional effect of the different practices and pickle compositions used in different establishments.

Although a large number of factors may affect the composition and quality of bacon, particular attention has been given to the effect of the number of "stitches" (injections) per side used in pumping, the number of days in cure, and the composition of the pickles used. This approach to the problem was favoured, as curing is fundamental to bacon production. The particular factors mentioned above are apparently considered to be the most important, since each plant adheres closely to its chosen practice respecting them. Again, if necessary to improve quality, these factors could be modified promptly and inexpensively.

The relation between pickle and product is a function of the scale on which the curing practice is conducted, as shown by the fact that small pieces of pork "cured" in the laboratory (7) may have a salt content more than twice that of the factory product. If the results of investigations into the relations between pickle and product are to be applied in practice, it is essential that the materials studied be typical of those used or produced on a commercial scale. The practices followed in the 22 plants represented in this study varied sufficiently to give considerable information on the effect of pickle composition and other factors on the quality of the bacon.

It was necessary to employ statistical methods to interpret the large body of data obtained, while retaining certain confidential details. For details of the statistical methods used in this and subsequent papers the reader is referred to Snedecor (8).

Method of Shipping Samples

Both pickle and bacon are subject to chemical and bacteriological changes if held at ordinary temperatures, and special arrangements had therefore to be made for transferring these materials relatively unchanged from the plants to the laboratory. The methods employed are described below.

Bacon

The period of transport from the plants to the port of Montreal varies from less than one to six days. To minimize abnormal changes during transport, the experimental sides were shipped with regular export shipments in a refrigerator car to the docks at Montreal. Immediately a car was opened, the samples were transferred to a room at 0 to 2° C. When the entire shipment of samples had been accumulated it was transferred to the laboratories by refrigerated truck.

Pickle

Effect of Age and Temperature on Composition

Before undertaking these studies it was necessary to determine the conditions under which pickle may be shipped for periods of about five days duration without significant change in its chemical composition or bacterial content. In some preliminary experiments tank pickles, taken at the beginning, middle, and end of cure, were stored at 1.1 or 4.5° C. and at 25° C. for periods of about a week, in small sealed jars under aerobic conditions, since air was almost certain to be present in the test samples. Analytical results indicated that the chloride and nitrate content remained constant, while the nitrite content and bacterial numbers showed greater variation from day to day than could be accounted for by experimental error. This led to a more extensive study of the changes in nitrite content and bacterial numbers of pickles obtained from one plant at various stages of cure.

The changes in the nitrite content of five of the above pickles, representing the several types of change observed during storage at 25 and 4.5° C. or lower, are shown in Fig. 1, and the changes in the bacterial content in Fig. 2. The points in these figures represent the means of the determinations made at each sampling, and the ordinate of the cross-hatched section the difference necessary for statistical significance computed from the standard error of the replicated tests.

The nitrite content was determined colorimetrically by a procedure already described (9). The curves on the left-hand side of Fig. 1 were plotted from values obtained by comparing the colour developed in the sample with that of a standard solution of nitrite in a visual colorimeter. Those on the right-hand side were obtained with a photoelectric colorimeter, which yielded more precise results as shown by the smaller necessary difference.

Bacterial numbers were determined on nutrient agar and on 10% salt agar with incubation at 20° C. In Fig. 2 the ordinate represents the changes from the initial value during storage, these changes being expressed as the change in the logarithm of the bacterial number per ml. of pickle. The number of organisms observed on the medium containing 10% salt was much higher than that observed on salt-free media. The actual number present was not of particular interest in this study but will be discussed more fully in later papers of this series.

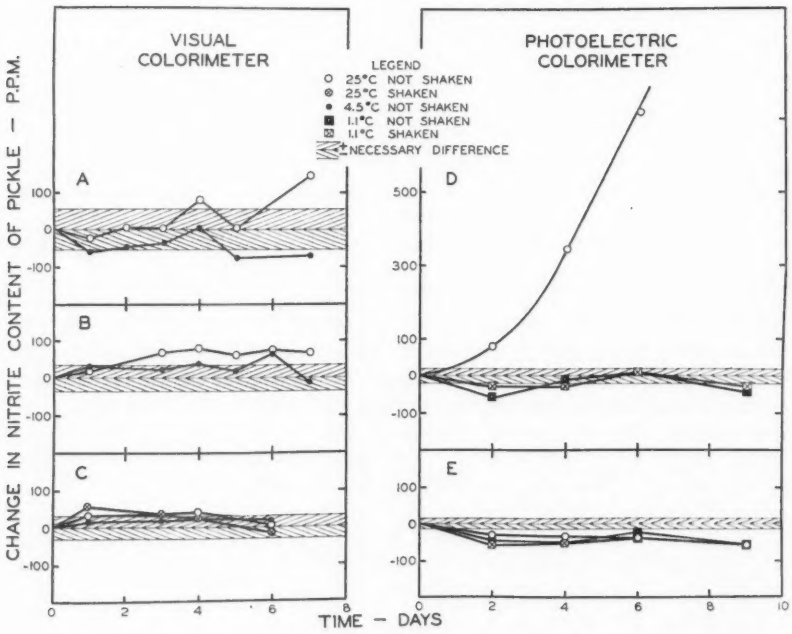

FIG. 1. *Changes in nitrite content of pickle during storage at 25° C. and 4.5° C. or lower.*

The curves in Fig. 1 show that storage temperature had little effect on the nitrite content of certain pickles (*C* and *E*), while others show a small (*A* and *B*), or decided (*D*), increase in nitrite content during storage at room temperature. Fig. 2 shows that the bacterial number observed on a salt-free medium remains relatively constant during storage at 4.5° C. or lower, but decreases during storage at 25° C. The counts observed on a medium containing 10% salt vary with time, but there is no consistent effect of temperature. These results showed definitely that the changes in nitrite content, and bacterial counts by certain methods, are reduced by keeping the pickle at a temperature of 4.5° C. or lower. Methods for maintaining these low temperatures during transport for periods up to five days will be described in the next section.

The results obtained with pickles *C*, *D*, and *E* (Fig. 1) and *D* and *E* (Fig. 2) indicate that shaking, comparable with that occurring in transport, had no appreciable effect on the nitrite content or bacterial numbers.

It is evident from Figs. 1 and 2 that even when the pickles are kept at temperatures of 4.5° C. or lower, the changes in nitrite content and bacterial numbers during storage may exceed the "necessary difference", or the day-to-day variation attributable to experimental error. The majority of these changes must therefore represent real alterations in composition. These

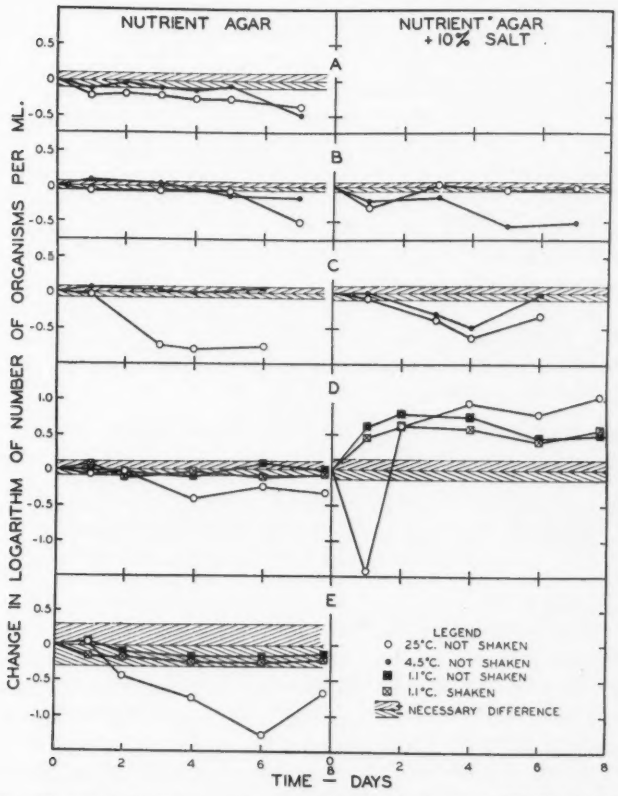

FIG. 2. *Changes in bacterial numbers in pickle during storage at 25° C. and 4.5° C. or lower.*

changes appear to be of two types: those showing a maximum or minimum during the storage period as shown by pickles *A* and *B* in Fig. 1, and *B* and *C* (10% salt medium) in Fig. 2; and those showing an increase or decrease only as shown by pickle *E* in Figs. 1 and 2. The analyses on these and other pickles failed to show any definite relation between the type of change and the stage of curing, i.e., whether the pickle was taken at the beginning, middle, or end of cure. Likewise the changes appear to be relatively independent of the original nitrite or bacterial content. It seems probable that some of the rather sudden changes in composition may be related to such phenomena as protein precipitation. It was therefore concluded that although pickles stored at low temperatures may show small but definite changes in composition, these changes depend on unknown properties of the individual pickle and are consequently unpredictable. Ingram's (5) conclusion that tank pickles may be shipped for periods up to 10 days at temperatures below 10° C.

without appreciable change in their nitrite content does not appear to apply generally.

The differential changes in different pickles during transport must therefore be recognized as an additional source of error, to be combined with the sampling and experimental errors. By obtaining two samples of pickle from each plant at different times, it was possible to compute an over-all estimate of the experimental error, errors due to differential changes during transport, and the error of sampling the tank. The significance of differences between the composition of pickles from different plants could therefore be assessed by comparison with the variance within plants at different times. It is possible that this latter value yields an exaggerated estimate of the error attributable to sampling, changes, etc., since it is almost certain that the composition of the original pickle from an individual plant varies, at least slightly, from time to time.

Although a decrease in the bacterial content of pickle, as indicated by growth on nutrient agar at 20° C., appears to be associated with an increase in nitrite content during storage at 25° C. in these experiments, little significance can be attached to this observation. The small containers used were by no means typical of the storage tanks employed in practice, and the experiments were not conducted on a sufficiently extensive scale to permit definite conclusions.

Containers for Maintaining Low Temperature During Transit

The results of the previous section showed the necessity for using containers that would maintain the pickles in the vicinity of the freezing point during shipment to the laboratories, i.e., for periods up to four or five days' duration.

Four types of containers were tested: (i) a common "thermos" bottle of half-pint capacity; (ii) an insulated commercial isothermal jug of one gallon capacity containing a half-pint jar of pickle and 7 lb. of ice; (iii) a gallon pail insulated with $1\frac{1}{2}$ to 2 in. of cork and containing $4\frac{1}{2}$ lb. of ice in addition to the pickle jar; and (iv) a 2-gal. can insulated with 2 to $2\frac{1}{2}$ in. of wool felt and containing 15 lb. of ice. Typical results given in Table I show that the last container only was satisfactory, and this type was used throughout the investigation. The majority of the experimental samples were received at 0° C., a few at 5° C., and only an occasional one at 10° C. or higher.

Method of Sampling

Bacon

The 44 sides of Wiltshire bacon, submitted by the 22 plants, were sampled for analysis three times: (i) on receipt at the laboratories, representative of their condition when shipped from Montreal; (ii) after 10 days' storage at 1.1° C., representative of their condition on arrival in London, England; and (iii) after smoking for 14 hr. at 43 to 46° C., which yielded material approximately representative of that in the British retail store. English practice favours smoking periods of 36 to 48 hr. at relatively low temperatures

TABLE I

TEMPERATURE IN °C. OF PICKLE KEPT IN CONTAINERS OF VARIOUS TYPES

Period of exposure at 25° C., days	"Thermos" bottle 1 pint capacity	Insulated commercial isothermal jug, containing ½ pint pickle + 7 lb. ice	Gallon pail insulated with 1½ – 2 in. cork dust, containing ½ pint pickle + 4½ lb. ice	2-gal. can insulated with 2 in. wool felt, containing ½ – 1 pint pickle + 15 lb. ice
0	2.3	0.2	0.0	0.0
1	12.5	1.1	0.0	0.0
2	17.8	16.7	1.0	0.0
3	21.0	—	11.1	0.0
4	22.5	—	19.7	0.0
5	—	—	—	12.5

compared with Canadian methods. (In these studies the ham was the only portion of the side to be smoked. For this and other reasons the smoking period was reduced.)

On receipt at the laboratory the sides were placed in a room at 4.5° C. and all sampling was done at this temperature. The sides were unwrapped and samples for determining the bacterial number taken first. After removal of material for the chloride, nitrate, nitrite, moisture, pH, colour, colour stability, and tenderness determinations, and measurement of the oxidation-reduction potential of the whole meat in the ham, the sides were rebaled and transferred to a room at 1.1° C. After 10 days' storage the bales were again opened and sampled for these determinations, with the exception of tenderness measurements. This completed the sampling for bacterial numbers, chloride, pH, and oxidation-reduction determinations, but the ham from each side was smoked and again sampled for the other determinations.

Samples were also taken from the sides for studies on salt distribution, the effect of heat treatments comparable with smoking, and the fats. The samples used for these experiments will be described in the papers reporting the results.

The exact position from which the samples were taken and certain details of sampling can best be described by reference to Fig. 3. Samples of the pleural membrane over the second and third, and over the ninth and tenth ribs were removed for making bacterial counts at each sampling. At the first sampling the fourth and fifth ribs were removed without contamination of the pleural membrane and stored separately in an atmosphere of 95% relative humidity at 1.1° C. for a longer period than the sides were stored, to determine the surface bacterial number when visible slime became evident.

Material for the other determinations was obtained after the ham was removed, as indicated in Fig. 3. Samples for chemical analysis and the pH measurements were obtained by cutting a slice from the ham, while a triangular section removed from the side provided material for colour, colour stability,

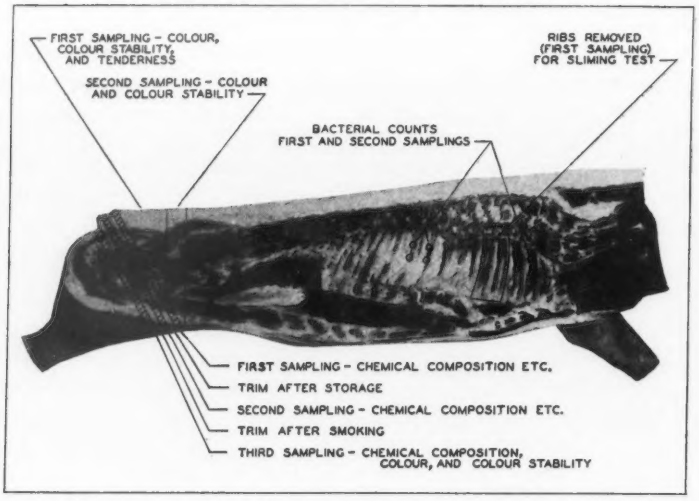

FIG. 3. *Portions of Wiltshire side used for determining bacterial counts, chemical composition, and physical properties at each sampling.*

and tenderness measurements. Oxidation-reduction potential was obtained by inserting a small pointed platinum electrode into the ham after removal of the slice for chemical analysis.

After storage the cut surface of ham was trimmed and another slice removed for analysis. At the same time a piece was removed from the side for colour and colour stability measurements. Oxidation-reduction potentials were again taken as described above. After smoking the ham was trimmed and another slice removed for both the physical and chemical measurements.

There are certain advantages and disadvantages in this more or less systematic method of sampling. Apart from certain practical limitations, it seemed desirable, since this study was concerned primarily with variations within and among plants, to take the samples from all the sides, at a given sampling, from comparable regions. This should permit more precise comparisons between the compositions of the same portion of different sides than if the samples had been taken at random from the whole side, or a preselected part of it. This procedure, however, sacrifices precision in determining the effect of ageing, for although the portions taken from each side at a given sampling are comparable, those taken at different samplings differ systematically in both time and position. In making the statistical analyses it is possible to determine the variance attributable to "between samplings" but it cannot be stated with assurance whether such differences represent a real change with time, or a difference between positions that existed originally. This subject will be discussed further in relation to the results obtained.

The slices of ham taken at each sampling for chemical analysis were immediately trimmed free of fat, bone, and connective tissue. Three small samples representing the outside, centre, and inside of the slice were removed for a study of salt distribution to be described in a later paper. The remainder of the lean meat was ground and thoroughly mixed by passing it through a food chopper several times. The pH of this ground material was immediately determined with a glass electrode, after which it was placed in a moisture-tight sample bottle, frozen in a room at −29° C. and held at this temperature until required for analysis. Storage in the frozen state usually did not exceed a few days. The samples were thawed by placing them in a room at 4.5° C. for a few hours. They were then mixed thoroughly and portions for analysis weighed out in the same cold room. The sample residues were refrozen immediately to preserve the material for any determinations that required to be repeated. Extreme care was necessary at this stage since ground material kept at 4.5° C. for more than a few hours showed a significant increase in nitrite content, and this change was accelerated by exposure to higher temperatures.

All the above measurements were objective, and no attempt was made to estimate subjective qualities such as flavour. A special study of this important attribute of quality has been undertaken.

Pickle

Two distinct pickles are used for curing Wiltshire bacon. One of these, designated "pump" pickle, is injected into the sides before they are placed in the curing vats, while the other, designated "tank" pickle, is used to cover the sides. Tank pickle suffers progressive changes in composition during the curing period. In order to obtain some estimate of the magnitude of these changes a sample of the tank pickle was taken when the sides were put in to cure, and again when they were removed. These two samples are subsequently designated "cover" and "spent" pickle respectively. When an estimate of the composition of tank pickle was necessary for certain computations, this was taken as the average composition of the cover and spent pickle with respect to the constituent in question.

Corresponding samples of pump, cover, and spent pickles were obtained twice from each plant with an interval of about a month between samplings. The second set of samples was taken from the pickles used for curing the bacon from which the experimental sides were obtained. In view of the small difference observed between successive pickle samplings, the mean composition of the two pickles was used for studying the relations between pickle and product in order to reduce the obscuring effect of experimental errors. The results of certain determinations on the pickles were lost or excluded as a result of errors in sampling, failures in transport, or losses in the laboratory.

When the pickle samples were received they were unpacked and placed in a room at 0° C. until the analyses were complete. Samples for bacterial numbers were removed to sterile glassware, plated, and incubated imme-

diately. Sufficient material for the chloride, nitrate, nitrite, pH, and oxidation-reduction potential measurements was removed to an ordinary laboratory for analysis and these determinations were made at room temperature. As the proteins tend to precipitate from pickle at room temperature the samples for this determination were pipetted from the original sample at 0° C. The remainder of the sample was filtered and colour measurements made in a room at 4.5° C.

Methods of Analysis

The methods employed for making the bacterial counts on both bacon and pickle will be described in later papers reporting the results of the measurements. The procedures followed in determining chloride, nitrate, and nitrite have already been described (9).

The protein nitrogen content of the pickle was determined on suitable portions (usually 10 or 25 ml. depending on the protein nitrogen content) pipetted into Kjeldahl flasks in the cold room. Nitrate nitrogen was removed (6, p. 27) before the Kjeldahl digestion.

The moisture content of the bacon was determined on 2- to 3-gm. portions of the ground material, by drying in flat aluminium dishes to constant weight (16 to 24 hr.) at 100° C. Preliminary measurements at lower temperatures yielded the same results, within experimental error, on this relatively fat-free material, but a much longer drying period was required. Actually it was difficult to attain a truly constant weight at any temperature, but after drying for 16 to 24 hr. at 100° C. the loss of weight over an additional 4-hr. drying period was never more than the equivalent of 0.05% moisture. Differences of this magnitude were smaller than the error between duplicates, and considerably less than the over-all sampling error.

The pH and oxidation-reduction potential measurements were made with a Bechmann pH meter. The appropriate standard electrodes supplied with the instrument were used for the determinations on pickles. The pH measurements on bacon were made in a room at 4.5° C., using a large glass electrode and extension leads. Both the glass and calomel electrodes were forced into a portion of the ground sample. Readings were required to check within 0.05 pH at different positions in the sample. Standard buffer solutions were used for adjusting the instrument at this low temperature, and all reported values were corrected to 20° C.

The oxidation-reduction potential of the bacon was measured with a special platinum electrode consisting of a 60° platinum cone, $\frac{1}{4}$-in. diameter at the base, carried on a $\frac{1}{4}$-in. diameter bakelite tube. This electrode was inserted to a depth of 2 to $2\frac{1}{2}$ in. into the ham and the circuit completed by inserting the calomel electrode into a cut at the surface. The observed potentials were converted to Eh by correcting for the potential of the calomel electrode at 4.5° C. The instrument and electrodes used for the Eh measurements were checked with a buffer solution of known pH containing quinhydrone. Several difficulties were encountered in making the Eh measurements in both bacon

and pickle, and there was some uncertainty in the results obtained. These will be discussed more fully when the results are presented in a later paper.

The colour and tenderness measurements on the bacon were made by means of instruments and methods already described (10, 11). These observations were made on slices or portions of whole meat, immediately after cutting, in a room at 4.5° C. Samples were chosen that were free from obvious streaks of fat or connective tissue. An estimate of colour stability was obtained by repeating the measurements on these slices periodically during exposure to air at a temperature of 10° C. and a relative humidity of 95%. This condition avoided serious drying of the samples, which has been shown to affect the colour (12). The results therefore indicate the stability of the colour to atmospheric oxidation at a temperature commonly prevailing in the storage chamber of a retail store.

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The authors wish to express their appreciation and thanks to the management and staffs of the packing companies for their cordial co-operation in providing certain confidential details relative to their curing practice, for donating the bacon and pickle for analysis, and for collecting and forwarding these samples. These include: Burns and Co. Ltd., at Calgary, Edmonton, and Prince Albert; Canada Packers Ltd., at Toronto, Peterborough, Hull, Montreal, and Edmonton; Dumarts Ltd.; F. W. Fearman Co. Ltd.; First Co-Operative Packers of Ontario; Fowler's Canadian Co.; Gainers Ltd.; J. M. Schneider Ltd.; Swift Canadian Co. Ltd., at Toronto, Winnipeg, Moose Jaw, and Edmonton; Wellington Packers Ltd.; Whyte Packing Co.; Wight and Co. Ltd.; and Wilsils Ltd. In addition the following firms furnished pickle and all other information requested but were unable to supply bacon at the time this phase was investigated: Burns and Co. Ltd., at Regina and Winnipeg; Canada Packers Ltd., Winnipeg; and Union Packing Co., Calgary. Special thanks are tendered to the management and staff of Canada Packers Ltd., Montreal, for assisting in the collection at Montreal of the bacon from all plants and its transport to Ottawa, and of Canada Packers Ltd., Hull, for smoking the test material and assisting with the sampling. It would have been impossible to conduct the investigation without the whole-hearted co-operation of these firms.

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CANADIAN WILTSHIRE BACON

II. CHLORIDE, NITRATE, AND NITRITE CONTENT OF BACON AND PICKLE[1]

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Abstract

Analysis of Wiltshire bacon produced in Canadian plants showed that different sides varied in their chloride, nitrate, and nitrite contents, the chloride content being the most uniform. Although the observed variations were statistically significant they do not necessarily affect the quality of the product. An analysis of variance showed that differences between the sides from different plants were the main general source of variation in the chloride and nitrate contents, while the differences between sides from the same plant were the main source of variation in nitrite content. This, and other evidence, indicates that the properties of the individual side affect its nitrite content to a considerable extent.

The variation in the composition of the pickles used in different plants was significantly greater than the variations within plants, although the chloride content was relatively uniform throughout. Other curing practices, such as the number of injections used for pumping a side and the time in cure, also varied between different plants. Statistical computations showed that the number of injections used for pumping was correlated with the chloride and nitrite content of the sides, while their nitrate content was correlated with the nitrate content of the pump pickle. Although these factors affected the composition of the product with respect to each constituent, the level of the correlation coefficients was rather low. It is therefore concluded that most of the observed variation in the bacon was contributed by other unmeasured factors, or by inherent differences between the carcasses.

The analysis of variance showed significant differences between the content of the three constituents at different samplings. The method of sampling, however, did not permit the true effect of ageing to be distinguished precisely from the effect of systematic differences in position, and the observed differences between samplings might possibly have been due entirely to the effect of position.

Introduction

Although the quality of bacon may be affected by many factors, relatively few of these can be closely controlled. Those that can be standardized and reproduced from time to time in a given plant include the composition of the pickles with respect to the several salts used, and certain of the curing processes. Different plants, however, use different pickle formulas and curing practices, and this, together with the fact that the composition of the product may depend on other factors that are not closely controlled, can give rise to certain variations in the product. This paper reports the chloride, nitrate, and nitrite contents of the bacon, the general sources of variation, and more specifically, the effect of pickle composition and certain curing practices.

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An outline of this investigation and a description of the methods used have been reported in the first paper (5) of this series. Statistical methods (7) were employed to reduce the large body of results, and permit their interpretation in terms of the required information.

The general mean and the standard deviation of a single observation served to describe the composition and over-all variation of the bacon and pickle with respect to each of the constituents. The coefficient of variability was computed from these quantities to facilitate comparison of the relative variability of different constituents. The practical significance of the observed variations had naturally to be assessed from other considerations.

The principal sources of variation were determined by an analysis of variance. For bacon, the total variance could be subdivided into portions attributable to: average differences between sides from different plants; differences between sides within plants; average differences between samplings; and sampling and analytical error. Although all the analyses of the samples were made in duplicate, it was impossible, within the limits of available time and material, to obtain and analyse two independent pieces from each side at each sampling. In consequence the residual variance was used to estimate the combined sampling and analytical error.

The results obtained with pickle were analysed in the same way except that only two sources of variance could be recognized, namely, within and between plants. For reasons already given (5) the variance within plants had to be taken as an estimate of the sampling and other errors. This variance probably overestimates these errors, since the samples for each plant were obtained at different times, and consequently the observed differences include any real change in the composition of the pickle from time to time.

The specific effect of pickle composition, number of stitches per side used in pumping, and the number of days in cure, on the concentration of salts in the product, was determined by computing simple correlation coefficients between the quantity of a given constituent in the bacon, the number of stitches per side, the number of days in cure, and the quantity of the same constituent in the pump and tank pickles. In some instances it was possible to combine two of these quantities. For instance the amount of salt contributed by the pump pickle may reasonably be taken as the product of its salt content and the number of stitches used per side. This assumption appears to be valid, since results by Callow (3, pp. 65–70) indicate that the amount of pickle retained is proportional to the number of stitches. Nevertheless some uncertainty remains, since the pumping equipment and method in different plants may not inject the same amount of pickle per stitch, while the number of stitches in the ham portion analysed may not remain in a fixed proportion of the number per side. Similarly the product of the chloride content of the tank pickle and the number of days in cure were used for computing certain correlations.

Finally the composition of the bacon will be affected by the composition and method of application of both the pump and tank pickles. The relative

effect of these two phases of curing cannot be established directly from simple correlation coefficients, since certain factors in each phase may be associated. Thus a plant using a weak pump pickle may also use a weak tank pickle, or a plant accustomed to the production of a mild bacon may reduce both the number of stitches used in pumping and the number of days in cure. By computing partial correlation coefficients, however, it was possible to determine the effect of the pumping practices independent of tank curing practices and vice versa. These computations were made wherever the simple correlations were significant, in order to establish the observed relation independent of associated factors.

Chloride

The mean chloride content of the bacon and pickles from all plants, over all samplings, appears in Table I. The average chloride content of 3.9% and a maximum of 5.8% indicate that Canadian Wiltshire is milder, on the average, than British bacon, if published results (1, 2) are typical of British curing practice.

The chloride content of the pickles is expressed on a weight-volume percentage basis, i.e., grams of sodium chloride per 100 ml. of pickle, or approximately lb. per 10 gal. The figures in Table I show that the mean chloride content of pump pickle approaches saturation (about 31.7% on a weight-volume basis at cellar temperature). Tank pickle is somewhat less concen-

TABLE I

CHLORIDE CONTENT OF BACON AND PICKLE

(As sodium chloride)

Statistic	Bacon	Pickle		
		Pump	Cover	Spent
Mean, %	3.93	29.20	28.32	23.80
Standard deviation, %	1.05	1.71	2.67	1.93
Coefficient of variability	26.79	5.86	9.41	8.10

Analysis of Variance

Variance attributable to	D.f.	Mean sq.	D.f.	Mean sq.	D.f.	Mean sq.	D.f.	Mean sq.
Sampling and analytical error	43	0.206	19	0.795	16	0.824	16	1.280
Between samplings	1	21.315**						
Between sides within plants	22	0.476*						
Between plants	21	2.652**	(18)	5.178**	15	13.815**	15	6.323**

* *Indicates 5% level of significance, in this and subsequent tables.*
** *Indicates 1% level of significance, in this and subsequent tables.*

trated initially and the chloride content naturally decreases during cure, cf., values for cover and spent pickles respectively. Although the greatest absolute variations in chloride content occur in cover pickle, the chloride content of the bacon is relatively more variable than that of the pickles.

The results of analyses of variance are given in the lower part of Table I. Those for bacon show that, on the average, both the variance between samplings from the same side and that between different sides from the same plant were significantly greater than the sampling and analytical errors. The variance between sides from different plants, however, was significantly greater than that between sides within plants. The significance of the variance between samplings will be discussed more fully in a later section. Although it must be recognized that sides cured at the same time in the same tank differ significantly in chloride content, it is obvious that the major portion of the variation is contributed by the different chloride contents of bacon from different plants.

The simple and partial correlation coefficients between the chloride content of the bacon and the chloride contents of the pump and tank (mean of cover and spent) pickles, the number of stitches per side used in pumping, and the number of days in cure, appear in Table II. The simple correlation coefficient between the chloride content of the bacon and the number of stitches used per side is the only one that exceeds the 5% level of significance. In fact, the coefficients between the chloride contents of the bacon and that of both pickles, although insignificant, are negative. This indicates that the chloride content of the bacon is not affected by the observed variations in the chloride content of the pickles used, but depends entirely on other factors.

TABLE II

COEFFICIENTS OF CORRELATION BETWEEN CHLORIDE CONTENT OF PICKLE AND CHLORIDE CONTENT OF BACON

(As sodium chloride)

Quantities correlated	Simple correlation coefficients	
	D.f.	r
Mean chloride content of bacon over all sides and samplings with:		
Pump stitches per side	20	0.45*
Days in cure	20	−0.33
Chloride in pump pickle (mean)	19	−0.36
Chloride in tank pickle (mean)	19	−0.10
Loss of chloride from tank pickle during cure (mean)	19	−0.33
Chloride in pump pickle (mean) × stitches per side	19	0.40
Chloride in tank pickle (mean) × days in cure	19	0.25
Chloride in pump pickle (mean) × stitches per side, independent of chloride in tank pickle (mean) × days in cure	18	0.41
Chloride in tank pickle (mean) × days in cure, independent of chloride in pump pickle (mean) × stitches per side	18	0.28

The simple and partial coefficients between the chloride in the product and such combined quantities as chloride in pump pickle times the number of stitches, and chloride in tank pickle times days in cure, were insignificant.

Although these results indicate that the number of stitches used for pumping a side is more important than any of the other factors studied, the level of the correlation coefficient, although significant, is so low that less than 20% of the observed variance in the chloride content of the bacon can be accounted for by variations in the number of stitches used. In consequence the major portion of the observed variation in chloride content, including that between sides from the same plant, must be due to unmeasured quantities concerned with curing, or to inherent differences between carcasses. Fortunately the over-all variation observed cannot be regarded as having a serious direct effect on the quality of the product, although it may be desirable to produce bacon of more uniform chloride content.

Nitrate

The mean nitrate content of the bacon and pickles, the standard deviation from these means, the coefficients of variability, and the results of an analysis of variance are given in Table III. The nitrate contents of the pickles are, on the average, comparable with those used in British practice (3, pp. 65–70) and would appear to be satisfactory. The nitrate content of both the product and the pickles is much more variable than their chloride content, as shown by the coefficient of variability. The relatively greater variation observed

TABLE III

NITRATE CONTENT OF BACON AND PICKLE

(As sodium nitrate)

Statistic	Bacon	Pickle		
		Pump	Cover	Spent
Mean, %	0.184	2.32	1.32	0.92
Standard deviation, %	0.220	2.469	0.923	0.644
Coefficient of variability	119	107	69.7	70.4

Analysis of variance

Variance attributable to	D.f.	Mean sq.	D.f.	Mean sq.	D.f.	Mean sq.	D.f.	Mean sq.
Sampling and analytical error	86	0.0125	19	0.342	16	0.380	16	0.056
Between samplings	2	0.0534*						
Between sides within plants	22	0.0228						
Between plants	21	0.2212**	18	12.173**	15	1.354**	15	0.798**

in the nitrate content of the bacon and pump pickle, as compared with that of the cover and spent pickles, was contributed largely by one plant that used much more nitrate than the average in its pump pickle. This practice had a direct effect on the nitrate content of the product.

The analysis of variance shows that there was a significant difference in the nitrate content between samplings. This phase will be discussed later. The difference between sides from the same plant did not exceed the analytical and sampling errors significantly. The difference between both the bacons and pickles from different plants, however, was significantly greater than the observed differences within plants.

The simple and partial correlation coefficients between the nitrate content of the bacon, and the known factors in the curing practice appear in Table IV. These correlation coefficients were computed on two bases, namely, including and excluding the results obtained from the plant using high nitrate concentrations in its pump pickle. By including the results from this plant, the correlation coefficients are based on the figures from which the results in Table III were computed. By excluding the results from this plant it was felt that the correlation coefficients were more typical of the effect of the ordinary variations in nitrate content of the pickles. As can be seen from the table, the exclusion of these results tends to reduce the degree of correlation between the nitrate content of the meat and quantities including the composition of the pump pickle, and to increase that with quantities involving the composition of the tank pickle.

TABLE IV

COEFFICIENTS OF CORRELATION BETWEEN NITRATE CONTENT OF PICKLE AND NITRATE CONTENT OF BACON

(As sodium nitrate)

Quantities correlated	Simple correlation coefficients			
	All plants		Exclusion of one exceptional plant	
	D.f.	r	D.f.	r
Mean nitrate content of bacon over all sides and samplings with:				
Pump stitches per side	20	0.13	—	—
Days in cure	20	0.29	—	—
Nitrate in pump pickle (mean)	19	0.92**	18	0.48*
Nitrate in tank pickle (mean)	19	0.59**	18	0.62**
Loss of nitrate from tank pickle during cure (mean)	19	0.09	—	—
Nitrate in pump pickle (mean) independent of tank pickle (mean)	18	0.88**	17	0.29
Nitrate in tank pickle (mean) independent of pump pickle (mean)	18	0.11	17	0.51*
Nitrate in pump pickle (mean) × stitches per side	19	0.89**	18	0.63**
Nitrate in tank pickle (mean) × days in cure	19	0.55**	18	0.57**
Nitrate in pump pickle (mean) × stitches per side, independent of nitrate in tank pickle (mean) × days in cure	18	0.80**	17	0.48*
Nitrate in tank pickle (mean) × days in cure independent of nitrate in pump pickle (mean) × stitches per side	18	0.02	17	0.39

Considering the coefficients given in the last column of Table IV it is evident that the nitrate contents of both the pump and tank pickles affect the nitrate content of the sides. This was to be expected, since the variation in the nitrate content of the two pickles was proportionately far greater than the methods used for their application. The partial correlation coefficients between the nitrate content of the bacon and that of the pump pickle, independent of tank pickle and vice versa, indicate that the nitrate content of the tank pickle has a greater influence on the composition of the product than that of the pump pickle. Nevertheless, the combined quantities, representing the known values of the pumping and tank-curing practices respectively, both yielded simple correlation coefficients that were highly significant. This suggests that the number of stitches per side and the number of days in cure, as well as the nitrate content of the two pickles, have some effect on the nitrate content of the sides. Partial correlation studies indicate that the composition of the pump pickle and the stitches per side are more influential than the composition of the tank pickle and the number of days in cure.

It is concluded from these results that the nitrate content of the pump and tank pickles and the number of stitches used in pumping are the principal factors affecting the nitrate content of the sides. It seems probable that, if more uniform pickle compositions and pumping practices were used in all plants, the relative variability of the product with respect to nitrate would be reduced to about the same level as that reported for chloride (Table I).

Nitrite

The mean nitrite content of the bacon and pickles, the variation of each, and the main sources of this variation are shown in Table V. The bacon con-

TABLE V

NITRITE CONTENT OF BACON AND PICKLE

(As sodium nitrite)

Statistic	Bacon	Pickle		
		Pump	Cover	Spent
Mean, p.p.m.	26.3	303	536	482
Standard deviation, p.p.m.	24.1	366	376	293
Coefficient of variability	91.6	121	70.2	60.7

Analysis of variance

Variance attributable to	D.f.	Mean sq.	D.f.	Mean sq.	D.f.	Mean sq.	D.f.	Mean sq.
Sampling and analytical error	86	127.8	19	8,331	16	6,551	16	3,966
Between samplings	2	818.6**						
Between sides within plants	22	1,213**						
Between plants	21	1.743	18	266,669**	15	285,593**	15	172,802**

tained 26 p.p.m. of sodium nitrite on the average. This is well within the 200 p.p.m. of sodium nitrite permitted by the Canadian pure food regulations (6). Although the coefficient of variability shows, on the average, considerable variation in the nitrite content of different sides, none of the sides, at any of the samplings, had a nitrite content approaching the legal limits.

The composition of the pickles with respect to nitrite content appears to be quite typical (3, p. 65–70), although these pickles were analysed during the summer months when the bacterial activity, and consequently the nitrite contents, would probably be maximal. On the whole the nitrite content shows about the same relative variability as the nitrate content.

The analysis of variance for bacon indicates that the difference between samplings and between sides from the same plant is responsible for most of the variance. In spite of the highly significant variations in the nitrite content of the pickles used in the different plants, the variance between the nitrite content of the bacon from different plants was not significant. This result is partly accounted for by the relatively large variance between sides within plants, most of which was contributed by the sides received from three or four plants. Detailed inquiry and examination of the results showed no valid reason for excluding these sides. The present investigation therefore indicates that the difference between sides treated in the same way is the main source of variation in nitrite content. More extensive analyses now under way, however, indicate that there is a significant difference between the nitrite content of sides from different Canadian plants after smoking in England. This cannot be taken as contradicting the present findings, since it will be shown later that the conditions used for smoking bacon in England may result in a differential development of nitrite in different sides.

The correlation coefficients between the nitrite content of the bacon and the known curing practices appear in Table VI. The correlation between the nitrite content of meat and the number of stitches used for pumping a side is the only one that is significant. Since correlations between the nitrite content of the bacon and that of the pump pickle never approach the level of significance, it appears that the number of stitches used for pumping increases the nitrite content of the bacon indirectly, rather than by a direct contribution of nitrite to the sides. This hypothesis is supported by the highly significant partial correlation between the number of stitches per side independent of nitrite in pump pickle. The possible nature of such an indirect effect is obscure, but it may result from the introduction of air or bacteria into the meat during pumping. It will be shown in a later paper that the pump pickles contained a considerable number of aerobic bacteria.

If nitrites can be produced from nitrates either on or within the sides after curing, it is evident that the observed nitrite content of the sides reflects both the extent or rate of this reaction as well as that between nitrite and haemoglobin, or muscle proteins. An attempt was therefore made to determine which of the two reactions was the more important in determining the nitrite content of the bacon. This was done by computing the nitrate : nitrite

TABLE VI

COEFFICIENTS OF CORRELATION BETWEEN NITRITE CONTENT OF PICKLE AND NITRITE CONTENT OF BACON

(As sodium nitrite)

Quantities correlated	Simple correlation coefficients	
	D.f.	*r*
Mean nitrite content of bacon over all sides and samplings with:		
Pump stitches per side	20	0.57**
Days in cure	20	0.29
Nitrite in pump pickle (mean)	19	0.25
Nitrite in tank pickle (mean)	19	0.25
Loss of nitrite from tank pickle during cure (mean)	19	0.21
Nitrite in pump pickle (mean) independent of stitches per side	18	0.21
Pump stitches per side independent of nitrite in pump pickle (mean)	18	0.57**
Nitrite in pump pickle (mean) independent of nitrite in tank pickle (mean)	18	0.15
Nitrite in tank pickle (mean) independent of nitrite in pump pickle (mean)	18	0.15
Nitrite in pump pickle (mean) × stitches per side	19	0.34
Nitrite in tank pickle (mean) × days in cure	19	0.23
Nitrite in pump pickle (mean) × stitches per side, independent of nitrite in tank pickle (mean) × days in cure	18	0.27
Nitrite in tank pickle (mean) × days in cure, independent of nitrite in pump pickle (mean) × stitches per side	18	0.12

ratio in the pickles and bacon from the individual plants. It was felt that if this ratio was significantly lower in the bacon than in the pickle, production of nitrite in the sides would be indicated, while a higher ratio in the bacon would suggest that reactions favouring the disappearance of nitrite predominated. Moreover, the use of the ratio would tend to minimize the effect of systematic variations in the composition of the meat between the different positions.

These ratios were found to be extremely variable both in the bacon and in the pickle. When they were subjected to an analysis of variance, neither the difference between samplings, between sides from the same plant, or between sides from different plants were significantly greater than the residual variance attributable to sampling and analytical error. The nitrate: nitrite ratio in the tank pickle only showed significant differences among plants. In fact, the mean ratio for the bacon over all samplings and sides did not differ significantly from that of the pickle over all samplings and plants.

It was observed, however, that two sides from each of two plants had a very low nitrite content at the first sampling, and since the nitrate content was about average, the nitrate : nitrite ratio was exceedingly large. The nitrite content at the later samplings, however, had increased considerably, indicating the production of nitrite within these sides. Since the different

sides in this group showed considerable variation in nitrite content at all samplings it was necessary to test the significance of the observed differences. For doing this the nitrate : nitrite ratio was used in preference to the nitrite content, for reasons already given.

Since the mean nitrate : nitrite ratio of these four sides varied widely both between sides and samplings, with the variance approximately proportional to the mean, it was necessary to use the logarithm of these ratios in making the analysis of variance in order to provide valid tests (4) of significance. The results of this analysis appear in Table VII, from which it is evident that the differences between sides and samplings are both significantly greater than the residual variance.

TABLE VII

ANALYSIS OF VARIANCE OF SAMPLES HAVING LARGE NITRATE : NITRITE RATIOS INITIALLY

Figures on basis of logarithms of ratios

Variance attributable to	Degrees freedom	Mean square
Between sides within times	3	2.01*
Between times (samplings)	2	2.62*
Residual	6	0.412

It is concluded from these results that sides having a high nitrate : nitrite ratio initially, i.e., low nitrite content, may increase in nitrite content during maturation and smoking. Furthermore, the difference between sides indicates that the extent of this increase depends on the properties of the individual sides. Whether the variable increases in the nitrite content of different sides were due to varying bacterial loads or to inherent differences in the carcasses themselves is not known. Since this behaviour was not observed over all samples it appears that under average conditions nitrite production does not occur to any significant extent in samples containing an average nitrite content initially.

Difference Between Samplings

Certain changes, termed maturation, occur in bacon after removal from cure. Although these are believed to be beneficial to the general quality and flavour of the product (3, pp. 70–72), their nature is obscure. It therefore seemed desirable to consider the changes that occurred in all of the individual constituents and properties measured. The results given in previous tables showed that the three constituents dealt with in this paper did differ significantly between samplings. However, as pointed out in the first paper (5), the difference between samplings includes the effect of systematic differences between the positions from which successive samples were taken as well as the true effect of ageing and smoking. Since certain constituents, such as chlorides, should not suffer any change with time, these can be used as reference substances for assessing the significance of observed differences in other constituents which might change, e.g., nitrites.

Table VIII shows the mean chloride, nitrate, and nitrite contents over all sides by samplings, the difference between these means, and the significance of the differences compared with the sampling and analytical error. All three constituents showed a significant increase between the first and second samplings. The chloride content was not determined at the third sampling. Between the second and third samplings, the nitrate content remained practically constant, and the nitrite content decreased significantly. The increased chloride content must represent the effect of position or chloride distribution, which will be discussed in a later paper. A similar conclusion must be reached for nitrate since there is no evidence that any appreciable quantity was converted to nitrite. Since the increase in nitrite between the first and second samplings is of the same order on a percentage basis as that observed with chloride and nitrate, it appears that this increase represents the effect of position rather than time. The decrease in nitrite between the second and third sampling may indicate a decrease in nitrite during smoking, since the nitrate content remains practically constant.

TABLE VIII

DIFFERENCE BETWEEN SAMPLINGS

Constituent	Mean by samplings			Difference between means		
	First (1)	Second (2)	Third (3)	1—2	2—3	1—3
Sodium chloride, %	3.43	4.42	—	0.985**	—	—
Sodium nitrate, %	0.144	0.204	0.205	0.060*	0.001	0.061*
Sodium nitrite, p.p.m.	22.6	31.1	25.2	8.42**	5.83*	2.59

A further attempt was made to determine the effect of age on nitrite content by computing simple correlation coefficients between the nitrite content and the age of the bacon at the three samplings independently, and over all analyses irregardless of samplings. These were found to be -0.04, -0.20, and -0.28, for the first, second, and third samplings respectively, and $+0.02$ over all samplings. None of these coefficients is statistically significant, although the negative sign within samplings indicates a decrease in nitrite with time. When the computation is made over all samplings, the effect of position intervenes, decreasing the correlation coefficient and changing its sign.

In conclusion there is no evidence to indicate that, on the average, a serious change occurs in the nitrite content of bacon during storage at 1.1° C., or smoking for 14 hr. at about 45° C. There is some indication that nitrite may be formed in sides having a low nitrite level on removal from cure. The difference between the nitrite contents of sides cured in the same plant suggests that much of the observed variability is due to some factor that is not closely controlled in commercial practice. This factor may be some inherent property of the individual sides, or differential bacterial contents.

Difference Between Bacon and Pickle from Different Plants

The results reported in earlier tables show that although the differences between sides from the same plant were generally significant, the differences between sides from different plants were usually greater. It is therefore of interest to determine how the concentrations of the several salts in the sides and pickles from the various plants are distributed around the general mean for each constituent.

Before preparing these frequency distributions, the difference necessary for statistical significance was computed for the variance between sides within plants for bacon, and from the variance within plants at different times for the pickles. These necessary differences were then used as the class interval in preparing the frequency distribution for each constituent. This method has the advantage of distributing the observed values over the number of classes that can be distinguished experimentally from one another, although individual results in adjacent classes may not differ significantly. It must be recognized, however, that a large number of classes will be distinguished if the material is variable with respect to the measurement in question, or if the variance between sides and pickles from a given plant is small, and vice versa.

The frequency distributions for the chloride, nitrate, and nitrite contents of the bacon and pickle appear in Fig. 1. Four classes can be distinguished in the bacon with respect to chloride content. Since the distribution around the mean is symmetrical, with only a few plants falling in the extremes, the

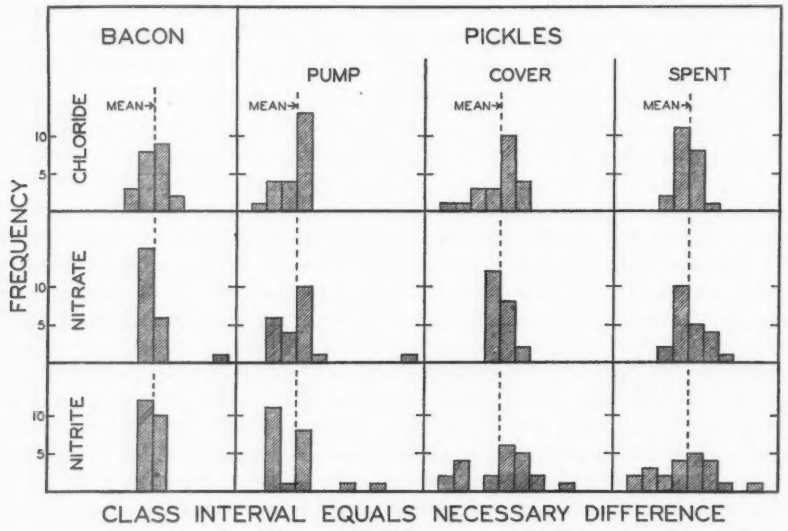

FIG. 1. *Frequency distributions of chloride, nitrate, and nitrite contents of bacon and pickle from different plants.*

chloride content of the product can be considered satisfactory. With the exception of the one plant using a high nitrate content in its pump pickle, only two classes can be distinguished with respect to the nitrate and nitrite contents of the bacon. This is due to the considerable difference observed between the content of these constituents in sides from the same plant.

The chloride content of the pump pickles falls into four classes, the distribution showing that the majority of the pickles are approximately saturated. Since the necessary difference for the pump and cover pickles was approximately the same, the more variable chloride content of the cover pickle is shown by a distribution over six classes. Only four classes of spent pickle could be distinguished.

The nitrate contents of the several pickles fall into three or five classes. Since the necessary differences were relatively large for this constituent, considerable variability is indicated. It has already been shown (Table IV) that the nitrate content of the pickles affects that of the bacon. It appears therefore that the use of pickles of more uniform nitrate content in the different plants would be desirable.

The nitrite content of the pickles was more variable than either the chloride or nitrate contents, since, in spite of the relatively large necessary differences, the values are scattered over about seven distinct classes. Although no direct relation could be demonstrated between the nitrite content of the pickle and bacon (Table VI), and in fact, there was some indication that the properties of the individual side determine its nitrite content, it would nevertheless seem desirable to standardize the nitrite content of the pickles used in the different plants as far as possible. In this connection it must be kept in mind that the combination of nitrite with the muscle pigments and proteins may occur differentially in different sides, and thus contribute to the observed variability.

In conclusion it should be pointed out that, although these results show statistically significant differences between the chloride, nitrate, and nitrite contents of bacon and pickle from different Canadian packing plants, it is not known that these variations seriously affect the final quality of the product.

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CANADIAN WILTSHIRE BACON

III. pH, OXIDATION-REDUCTION POTENTIAL, AND MISCELLANEOUS MEASUREMENTS ON BACON AND PICKLE[1]

By W. H. Cook[2] and A. E. Chadderton[3]

Abstract

The pH of bacon was relatively uniform, but it was possible to demonstrate statistically significant differences between sides cured in the same plants. Sides from different plants were however no more variable than those from the same plant. The results indicate that the pH of the bacon is affected by the pH of the pump pickle, decreases with the time in cure, and increases with the age from cure.

The absolute values of the Eh potentials observed in bacon were doubtful, but since the measurements indicated a statistically significant difference between sides from different plants it appears that this property may be a function of curing practice. Although the moisture content of bacon was relatively uniform, there was a significant difference between sides from different plants, and a significant loss of moisture during maturation and smoking.

The protein content of the tank pickle from different plants varied considerably, and probably reflects the effect of different handling practices. Nevertheless it was possible to demonstrate a direct relation between protein content and pH of the pickle.

Introduction

This paper constitutes one of a series covering an investigation of factory-cured Wiltshire bacon. An outline of the complete investigation and the methods employed were reported in the first paper (7). Of the many factors that may affect the quality of bacon, only a limited number can be controlled in commercial practice. For instance, the salt, nitrate, and nitrite contents of the pickle, and certain curing practices, such as the method of pumping sides, and the curing time, are ordinarily controlled and standardized within a given factory. This gives some control over the composition of the product with respect to the curing salts. These constituents and practices have been dealt with in an earlier paper (8). Other factors which are not controlled to any extent in either the pickle or the product, except indirectly, may also have some influence on the quality of the bacon. These include the pH and oxidation-reduction potential of the pickle and product, the protein content of the pickle, and the moisture content of the bacon. This paper deals with measurements of these properties in bacon and pickle from different packing plants.

Certain evidence (4, 5, 9) indicates that the pH of both the pork and the pickles may affect the final quality of the bacon with reference to its water and salt content, colour, and subsequent taint development. Indirect inform-

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ation suggests that the oxidation-reduction potential may influence the colour. Brooks (2) has shown that, even in the absence of oxygen, the reaction between haemoglobin and nitrite yields a mixture of both nitrosohaemoglobin and methaemoglobin, and that the presence of a reducing agent is required to prevent the formation of the latter compound. Since methaemoglobin is not found to any extent in the interior of bacon, it follows that the tissues contain some reducing substance having an oxidation-reduction potential lower than that of haemoglobin-methaemoglobin (6).

Other results (3) indicate that the moisture content of the lean part of the bacon decreases as the salt content increases, even after due allowance has been made for the salt present. The protein content of the tank pickle may affect other properties, for example, rate of bacterial development and consequently nitrite production, and thus indirectly affect the quality of the final product. The results of these miscellaneous measurements are reported in this paper. The interrelations between the several constituents in, and properties of, the product will be discussed in a later article.

The detailed observations on the several properties were reduced by statistical treatment (12) and are presented in the same form as that used in an earlier paper (8).

Hydrogen Ion Concentration

The mean pH values over all samples of bacon and pickle, and their standard deviations on a single observation basis, appear in Table I. The results show that the pH of the bacon from the various sources was remarkably uniform, with a mean value of pH 5.74. In general, the pump pickles were

TABLE I

pH OF BACON AND PICKLE

Statistic	Bacon	Pickle		
		Pump	Cover	Spent
Mean	5.74	7.86	6.80	6.42
Standard deviation	0.14	0.58	0.67	0.21

Analysis of variance

Variance attributable to	D.f.	Mean sq.	D.f.	Mean sq.	D.f.	Mean sq.	D.f.	Mean sq.
Sampling and analytical error	43	0.0064	18	0.291	16	0.270	16	0.048
Between samplings	1	0.1023**						
Between sides within plants	22	0.0264**						
Between plants	21	0.0371	17	0.379	15	0.646*	15	0.040

* *Indicates 5% level of significance in this and subsequent tables.*
** *Indicates 1% level of significance in this and subsequent tables.*

alkaline, having a mean pH of 7.86. The tank pickle was slightly acid initially and became more acidic during cure. Even at the end of cure, however, it was still more alkaline than the sides. The variability in the pH of the pickles appears to be related to their protein content and buffer capacity, since spent pickle, having the highest protein content (Table V), is the least variable.

The results of an analysis of variance appear in the lower part of Table I. In spite of the small over-all variation in the pH of the bacon, the differences between samplings and between sides from the same plant were highly significant, while the differences between sides from different plants were not significantly greater than those observed within plants. Other investigations (4, 5, 11) have shown that there is considerable variability in the pH of pork. The findings suggest that the small variations in the pH observed in bacon may be due primarily to differences in the pH of the pork.

The difference between the pH of the pickles from different plants was significantly greater than the variations within plants for the cover pickle only. There was some evidence that there was a significant variation in the pH of the pump pickle in a given plant from time to time, but for reasons already given (7) it was impossible to distinguish precisely between variability originating from this source and that attributable to sampling and other errors.

Simple correlation coefficients were computed between the mean pH of the bacon over all samplings and the pH of the pickles and other known curing practices, as outlined in Table II. The three significant correlation coefficients obtained show that the pH of bacon is inversely associated with the days in cure and with the pH of the tank pickle × days in cure, and directly related to the pH of the pump pickle. It appears that the number of days in cure is primarily responsible for the significance of the combined quantity.

TABLE II

SIMPLE AND PARTIAL COEFFICIENTS OF CORRELATION BETWEEN THE pH OF PICKLE AND pH OF BACON

Quantities correlated	D.f.	r
Mean pH of bacon over all sides and samplings with:		
Pump stitches per side	20	0.00
Days in cure	20	−0.52*
pH of pump pickle (mean)	20	0.46*
pH of tank pickle (mean)	20	−0.09
pH of pump pickle (mean) independent of pH of tank pickle (mean)	19	0.49*
pH of tank pickle (mean) independent of pH of pump pickle (mean)	19	−0.20
pH of pump pickle (mean) independent of days in cure	19	0.32
Days in cure independent of pH of pump pickle (mean)	19	−0.41
pH of pump pickle (mean) × stitches per side	20	0.09
pH of tank pickle (mean) × days in cure	20	−0.50*

Partial correlative coefficients between the pH of the bacon and the pH of the pump pickle independent of days in cure, and days in cure independent of pH of pump pickle, were both insignificant. These results show that although both these factors influence the pH of the bacon, it cannot be said which is the more important.

Certain supplementary studies were therefore made to obtain more definite information. It was found that while the pH of pork was scarcely affected by immersion in pickles at pH 5.0 to 5.5 for periods of a day or two, the injection of relatively small quantities of acidic but unbuffered brines, comparable with pump pickle, had an immediate and permanent effect on the pH of the pork. This suggests that if it is desirable to modify the pH of pork by means of the pickle, acidification of the pump, rather than the tank pickle would be the more effective method.

It is known that the pH of pork varies considerably (4, 5, 11). Unpublished results indicate that under Canadian conditions it may range from pH 5.5 to 6.5. Since the pH of the bacon was 5.7 (Table I), it appears that the meat must have become more acidic during cure. This could scarcely have resulted directly from the injection or absorption of pickle, since the pickles were generally alkaline to pH 5.7. These considerations suggest that the extent of the reaction affecting the pH of the bacon is affected by the length of the curing period independent of the pH of the curing pickles.

Oxidation-reduction Potential

The methods employed for measuring the oxidation-reduction potential of both the bacon and pickle have already been described (7). These results were subject to some uncertainty for the following reasons: (i) the potentials observed in both bacon and pickle showed considerable "drift" with time, and although the readings were not accepted until equilibrium had apparently been established, it is probable that errors of the order of 5 to 10 mv. occurred in some samples; (ii) since the pickle samples were exposed to air during transport, sampling, and measurement, it seems likely that the observed potentials were somewhat higher than those occurring in the curing tanks; (iii) for bacon two apparently identical electrodes gave, on the average, widely different absolute potentials.

In spite of these uncertainties the results are reported in Table III, since certain deductions are possible. The values reported for bacon were obtained with the No. 1 electrode, which gave a mean potential of about 24 mv. as compared with a value of about −121 mv. obtained with the No. 2 electrode on the same samples. The results obtained with the No. 1 electrode are preferred since it reproduced, from time to time, the correct potential in a quinhydrone-buffer solution more closely and consistently than the No. 2 electrode.

All three pickles were found to have about the same mean potential. It seems probable that the observed values represent the actual potentials in

TABLE III

OXIDATION-REDUCTION POTENTIAL (Eh) OF BACON AND PICKLE

Statistic	Bacon (Electrode No. 1)	Pickle		
		Pump	Cover	Spent
Mean, mv. (+)	23.7	338	328	328
Standard deviation, mv.	89.9	31.8	30.2	35.6

Analysis of variance

Variance attributable to	D.f.	Mean sq.	D.f.	Mean sq.	D.f.	Mean sq.	D.f.	Mean sq.
Sampling and analytical error	31	2,489	18	975	16	554	16	628
Between samplings	1	9,025						
Between sides within plants	22	1,636						
Between plants	21	22,422**	17	1,044	15	1,294	15	1,952*

the pickles as received, as they were reproducible, within the error attributable to "drift", with different electrodes. Since these samples had suffered exposure to air, however, there is some doubt that they represent material at all comparable with that existing in a curing tank.

In order to obtain information on this point, the oxidation-reduction potential was measured daily at four positions in a tank during cure. The four positions represented two depths, 4 in. and 3 ft. 10 in. below the surface, at two places in the tank. These readings varied considerably with position, depth, and time, and further information is required before a definite statement can be made as to the effect of these variables. It is sufficient to point out that the maximum, minimum, and mean values observed were 245, −189, and 86.2 mv. respectively. Since these values are considerably lower than those reported for tank pickle in Table III, it would appear that a satisfactory estimate of the oxidation-reduction potential can only be obtained on samples that have not suffered undue exposure to air.

Although the observed potentials in the main series of experiments may not be absolute, the results of an analysis of variance are of interest. The difference between plants, for bacon and spent pickles, was the only statistically significant source of variance, distinguishable from experimental error. In this respect both the electrodes used in bacon yielded similar results. It appears from this that the oxidation-reduction potential of bacon is dependent on the handling and curing practices followed in a particular plant.

Because of the uncertainty of the results, only a few correlation coefficients were computed. The coefficient between the Eh of bacon (No. 1 electrode) and that of the tank pickle (mean of cover and spent) was insignificant ($r = 0.25$ for 19 degrees of freedom). Although the Eh of both spent pickle

and bacon differed significantly between plants, they were not significantly correlated ($r = 0.11$).

The results presented in an earlier paper (8) showed that the nitrite content of the bacon was related to the number of stitches used in pumping, independent of the nitrite content of the pump pickle. It was felt that this might be the result of the introduction of air or bacteria with the pump pickle. Since the introduction of air might affect the oxidation-reduction potential of the bacon, the correlation coefficient between this quantity and the number of stitches per side was computed. The insignificant value ($r = 0.03$) was obtained. This does not necessarily disprove the above hypothesis, as the measurements on the bacon were made a considerable time after the pumping operation.

Moisture Content of Bacon

The moisture content of the bacon was determined at all three samplings. The results, including the analysis of variance, appear in Table IV. The mean moisture content over all sides and samplings was 71.38% with a relatively small variation. Nevertheless, an analysis of variance showed that the differences between samplings and between sides from different plants were highly significant. The loss of weight during storage and smoking doubtless accounts for the differences between samplings, a subject to be discussed further in a later section of this paper. The differences between the moisture content of the sides from different plants may be the result of the differences in salt content (3). These relations will be presented in a later paper.

TABLE IV

MOISTURE CONTENT OF BACON

Statistic	
Mean, %	71.38
Standard deviation, %	1.58
Coefficient of variability	2.22

Analysis of variance

Variance attributable to	D.f.	Mean sq.
Sampling and analytical error	86	0.621
Between samplings	2	93.968**
Between sides within plants	22	0.970
Between plants	21	3.100**

Protein Nitrogen Content of Pickle

The results of the Kjeldahl nitrogen determinations appear in Table V. Although expressed as the percentage of protein nitrogen this determination also includes lower compounds which yield ammonia on digestion. The mean protein nitrogen content of the pump pickle was 0.0008%. In some

instances, however, the samples contained almost as much as certain cover pickles. No statistical computations were made on the results obtained with pump pickles. The results in Table V show that the mean protein nitrogen content of cover pickle was about 0.09%, while that for spent pickle was 0.12%. The analysis of variance shows that the protein content of the pickles from different plants differs significantly, and probably reflects the effect of the different handling, treatment, or storage practice.

TABLE V

PROTEIN NITROGEN CONTENT OF PICKLE

Statistic	Cover	Spent
Mean, %	0.092	0.121
Standard deviation	0.049	0.040
Coefficient of variability	53.5	33.2

Analysis of variance

Variance attributable to	D.f.	Mean sq.	D.f.	Mean sq.
Sampling and analytical error and variance between samplings	16	0.00032	16	0.00053
Between plants	15	0.00467**	15	0.00278**

Although the observed variations in tank pickle could be accounted for in this way, an attempt was made to determine whether the solubility of the protein was dependent on any other properties of the pickle. The computation of correlation coefficients between the protein content and the pH and salt content of the pickle yielded values of 0.63 (highly significant), and 0.25 (not significant), respectively. It is therefore concluded that, whereas the observed variations in the salt content of the pickle (8) have no effect, the solubility of the proteins increases with increase in pH within the range (Table I) experienced in practice. This latter finding is in general agreement with the results of other investigations (11).

Difference Between Samplings

It has already been pointed out (7) that the design of the experiment did not permit accurate estimation of the effect of time, as the observed difference between samplings included both systematic differences between the positions from which successive samples were taken, and the true effect of ageing. The results obtained with salt and nitrate (8) led to the conclusion that a systematic difference between positions did exist. In consequence the observed differences between the nitrite contents at the several samplings had to be attributed to the effect of position rather than time. The question therefore arises as to whether the observed changes in the pH and moisture content of the bacon

represent a real change with time or merely variation between different positions.

The mean pH and moisture content of the bacon at each sampling is given in Table VI. These results show that both the pH and moisture content decreased significantly between successive samplings. Since evaporation is to be expected during storage and smoking, the decrease in moisture content doubtless indicates a real effect of time rather than the influence of position.

TABLE VI

DIFFERENCES BETWEEN SAMPLINGS IN pH AND MOISTURE CONTENT OF BACON

Constituent or property	Mean by samplings			Remarks
	First	Second	Third	
pH	5.78	5.71	—	Differences between means highly significant
Moisture, %	72.83	71.39	69.91	Differences between any two means highly significant

The decrease in pH between samplings, although significant, is rather small, and might have resulted from the influence of position rather than a real change with time. Earlier results (Table II) indicate that the pH of bacon may be affected by the time in cure, and it is reasonable to believe that the pH might also be affected by the time from cure. The difference between the mean pH values at the different samples is not a satisfactory method of studying the effect of ageing, since the sides varied in age from 2 to 11 days at the time of the first sampling. Consequently, any change that takes place with time might reasonably have occurred to various extents in the sides, quite apart from the uncertainties arising from the effect of position.

In order to obtain more definite information on the effect of time, at various stages, on the pH of bacon, simple and partial correlation coefficients were computed between the pH of the bacon and the elapsed time before, during, and after cure. By making these computations separately for each sampling, the possible effect of differences between the positions from which successive samplings were taken was excluded. The results appear in Table VII.

Since the correlation coefficient between pH and days from slaughter to cure was not significant, it appears that the ordinary variations in cooling time have no effect on the acidity of the smoked bacon.

Since the pH of the pump pickle and the period in cure have been shown to affect the pH of bacon to some extent, it might be that the length of holding period prior to cure had an effect on the pH of pork which was subsequently altered by other factors. However, the results of investigations on rabbit and poultry muscle (1, 10), and some unpublished results on pork, indicate that the ultimate pH of muscle tissue is attained well within the shortest cooling period used in practice.

TABLE VII

SIMPLE AND PARTIAL COEFFICIENTS OF CORRELATION BETWEEN pH OF BACON AND TIME IN CURE AND AGE FROM CURE

Quantities correlated	D.f.	r
pH of bacon—first sampling with:		
Days from slaughter to cure	15	−0.01
Days in cure	20	−0.53*
Days from cure to first sampling	19	+0.05
pH of bacon—second sampling with:		
Days in cure	19	−0.39
Days from cure to second sampling	19	+0.53*
pH of bacon—first and second samplings with:		
Days from cure when measurement made for both samplings	40	−0.09
pH of bacon—first sampling with:		
Days in cure, independent of days from cure to first sampling	18	−0.51*
Days from cure to first sampling, independent of days in cure	18	0
pH of bacon—second sampling with:		
Days in cure, independent of days from cure to second sampling	18	−0.40
Days from cure to second sampling, independent of days in cure	18	+0.53*

The correlation coefficients between pH and time in cure were negative, and significant at the first, but not at the second sampling. The coefficients between pH and time from cure were positive and significant only for the second sampling. Similar results were obtained when the opposing effects of time in cure and time from cure, were rendered independent by partial correlation. This indicates that the pH of bacon decreases during cure but increases during maturation.

The increase in the pH of bacon during maturation was confirmed by the results of a supplementary experiment in which ground samples of bacon were stored at 1 to 2° C. for some time. The final pH values attained varied from 6.0 to 8.3. It was also observed that the colour and final pH of the bacon appeared to be related. All samples at about pH 6.0 were grey, those at pH 7.0 greyish-brown to brown, while those at pH 8.0 or higher had retained their red colour. A further investigation of the effect of pH on colour change has been projected.

Difference Between Bacon and Pickle from Different Plants

In a previous paper (8) dealing with the chloride, nitrate, and nitrite contents of bacon and pickle, it was found that the differences between plants were usually the major source of variation. The properties and constituents considered in the present paper did not show such marked variation between plants. With respect to pH, significant differences between plants could be demonstrated for cover pickle, but not for the pump or spent pickle, or for the bacon. The oxidation-reduction potential measurements showed significant differences between the bacon and spent pickles from different plants.

The different practices followed in the different establishments were reflected by highly significant differences in the moisture content of the bacon, and the protein content of the cover and spent pickles.

The generalizations summarized in the above paragraph are evident from the results presented in earlier tables. Since individual plants seldom differed greatly from the general means, and since the factors considered in this paper could only be controlled indirectly, more detailed discussion of this source of variation is considered unnecessary.

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CANADIAN WILTSHIRE BACON

IV. CORRELATION BETWEEN CONSTITUENTS AND PROPERTIES OF BACON[1]

By W. H. Cook[2] and W. H. White[2]

Abstract

Simple correlation coefficients computed between several observed properties and constituents of factory-cured Wiltshire bacon showed the following to be associated: nitrate and chloride contents; nitrite and chloride contents; nitrite content and pH; and moisture and chloride contents. The first two of these associations probably arise from the pickle compositions and curing practices followed in the plants. The last two associations suggest a certain degree of dependence between the two properties, i.e., the nitrite content of the meat increases with the pH, and the moisture content decreases as the chloride content increases. When the moisture content of the bacon was expressed on a salt-free basis, the correlation between the moisture and chloride contents was not significant. This indicates that curing practices favouring a high salt content do not result in the removal of more moisture from the sides.

Introduction

The chloride, nitrate, nitrite, and moisture contents, pH values, and oxidation-reduction potential of factory-cured Wiltshire bacon and the pickles used for its manufacture, have been reported in earlier papers of this series (3, 4). These papers also reported the degree of correlation between similar constituents in the pickle and product. This paper deals with the correlations between the measured properties of the bacon.

Procedure

The methods employed for making these measurements have already been described (5, 8). The computation of correlation coefficients (7) between the observed quantities over 44 sides, representing 2 sides from each of 22 packing plants, served to determine whether statistically significant correlations existed between the measurements. It must be recognized, however, that a significant correlation between two quantities merely demonstrates that they are associated, and does not necessarily indicate that one is dependent on the other. Even where it seemed reasonably certain that one quantity was dependent on another, it was frequently difficult to determine which of the two was the causal agent. Thus a highly significant negative correlation between the pH and salt content of bacon could indicate either that the salt penetration was more rapid in the more acid sides, or that the pH decreases as the salt content increases.

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Results

Simple Correlation Coefficients

The simple correlation coefficients between these quantities appear in Table I. These coefficients were computed from the results obtained for each sampling at which the measurements were made, and also between the mean values for each constituent over all samplings for each of the sides analysed. This procedure was followed because certain of the observed differences between samplings were attributable to the differences between positions (4, 5) from which successive samples were taken, while others may have represented a real change with time. In consequence it seemed desirable to compute the correlation coefficients from the results obtained at each sampling independently. On the other hand, the variations between different sides for certain properties were small. In such instances it was felt that the variance attributable to over-all sampling and experimental error for any two properties being studied might be sufficiently large to mask any existing correlation. Consequently it was desirable to use in addition the mean values for each measurement over all samplings in order to reduce the variance attributable to sampling and analytical error.

TABLE I

SIMPLE CORRELATION COEFFICIENTS BETWEEN OBSERVED CONSTITUENTS AND PROPERTIES OF BACON

(Degrees of freedom = 42, for all except bracketted value = 30. Chloride, nitrate, and nitrite as the sodium salts)

Quantities correlated	Sampling			
	First	Second	Third	Mean of all
Nitrate in bacon with:				
Chloride in bacon	0.30*	−0.26	—	0.40*
Nitrite in bacon with:				
Chloride in bacon	0.45**	0.28	—	0.38*
Nitrate in bacon	−0.05	−0.23	−0.04	−0.07
Moisture in bacon	−0.04	−0.04	−0.06	−0.02
pH of bacon	0.56**	0.30*	—	0.47**
Eh potential of bacon (El. 1)	—	0.13†	—	0.16
Eh potential of bacon (El. 2)	[0.10]†	0.37*†	—	—
Moisture in bacon with:				
Chloride in bacon	−0.56**	−0.46**	—	−0.60**
Nitrate in bacon	−0.48**	−0.06	−0.07	−0.25
pH of bacon	0.18	0.17	—	0.13
Eh potential of bacon (El. 1)	—	0.05	—	—
pH of bacon with:				
Chloride in bacon	0.09	−0.23	—	−0.04
Nitrate in bacon	0.04	−0.05	—	—
Eh potential of bacon (El. 1)	—	—	—	0.26

† *Correlated with mean nitrite content over all samplings.*

* *Indicates 5% level of significance.*

** *Indicates 1% level of significance.*

It is evident that values for a number of possible correlation coefficients do not appear in Table I. Most of these omissions result from the fact that several of the determinations were not made at the third sampling. Since the observed oxidation-reduction potentials of the bacon are subject to some uncertainty for reasons already given (3), only a few computations relating this quantity to other properties were made.

The majority of the correlation coefficients reported in Table I are not significant and do not require comment. Significant positive correlations were obtained between the chloride and nitrate contents of the bacon at the first sampling and between the means over all samplings. These coefficients doubtless reflect the association between the concentration of these two substances in the curing pickles or between the curing practices used in different plants, and are therefore of little consequence.

A significant positive correlation was obtained between the nitrite and chloride contents at the first sampling, and between the mean nitrite and chloride contents over all samplings. There was also a significant positive correlation between the nitrite content and the pH of the bacon at all samplings.

Significant negative correlations were obtained between the moisture and chloride contents at all samplings, and between the moisture and nitrate contents at the first sampling. The only significant correlation involving the oxidation-reduction potential was obtained with nitrite at the second sampling, the No. 2 electrode being used. Since these measurements were uncertain, and those with Electrode 2 probably less reliable than those with Electrode 1 (3), further comment is unnecessary.

Partial Correlation Coefficients

The relation between the nitrite content and the pH and chloride content, and that between the moisture content and the chloride and nitrate contents, were investigated further by computing partial correlation coefficients between these quantities. The values obtained appear in Table II. The correlation coefficients between the nitrite and chloride contents, independent of pH, were positive and highly significant at both the first and second samplings independently, and also between the mean values for each side over all samplings. There is therefore little doubt that the nitrite content of these sides increased with their chloride content. It seems probable, however, that these two constituents are not dependent on one another but are merely associated through the pickle compositions or curing practices followed in the different plants.

The coefficients representing the degree of correlation between the nitrite content and pH, independent of chloride content, also indicate a definite association between these quantities, the nitrite content increasing as the pH increases. Certain evidence indicates that the pH of pork (2, 6) and the nitrite content of the bacon (4) are properties of the individual side and to that extent are independent of the curing practices followed. Consideration of these facts sug-

TABLE II

PARTIAL CORRELATION COEFFICIENTS BETWEEN OBSERVED CONSTITUENTS AND PROPERTIES OF BACON

(Degrees of freedom = 41. Chloride, nitrate, and nitrite as the sodium salts)

Quantities correlated	Sampling		
	First	Second	Mean
Nitrite in bacon with chloride in bacon independent of pH of bacon	0.48**	0.59**	0.45**
Nitrite in bacon with pH of bacon independent of chloride in bacon	0.59**	0.39**	0.53**
Moisture in bacon with chloride in bacon independent of nitrate in bacon	−0.50**	−0.49**	−0.56**
Moisture in bacon with nitrate in bacon independent of chloride in bacon	−0.39**	−0.21	−0.01

** *Indicates 1% level of significance.*

gests that there may be a dependence of one of the properties on the other rather than a mere association. If this is so it seems likely that the nitrite content is dependent on the pH rather than the reverse, since it is highly improbable that the small quantities of nitrite present would have any effect on the pH of the bacon. Such a dependence could be explained in several ways, namely: a more rapid penetration of the nitrite from the tank pickle as the pH of the meat increases; a more rapid production of nitrite from nitrate at higher pH levels; or a decreased rate of combination of nitrite with the muscle proteins and pigments at high pH levels. It has been shown that a high electrical resistance is associated with a high pH, and a slow penetration of chloride (2). If the absorption of nitrites is comparable with that of chlorides it seems unlikely that the first explanation is adequate for the observed differences in pH. Likewise it would appear that the rate of combination of nitrite with the muscle pigments would not be affected appreciably (1) by the variations in pH at the levels observed (3) in these sides. On the other hand the rate of reduction of nitrates by bacterial activity might reasonably proceed more rapidly as the pH increased within the observed range.

The results in Table II show that the correlations between moisture and chloride contents of the bacon, independent of nitrate content, were highly significant at each sampling independently, and for the mean values over all samplings. These show quite definitely that the moisture content decreases as the chloride content decreases. The correlation between the moisture and nitrate contents, independent of chloride content, was significant only for the results of the first sampling. It appears therefore that although the nitrate content may have a slight independent effect on the moisture content of the bacon, it is of secondary importance compared with that of chloride.

Relation between Moisture and Chloride Contents

The decrease in the moisture content with increase in chloride content of the whole bacon may merely reflect the effect of the additional dry matter present as sodium chloride. On the other hand it may be due to this effect plus the influence of certain curing practices, if conditions favouring the absorption of greater quantities of chloride also favour the loss of additional quantities of moisture from the sides. In order to eliminate the direct effect of the chloride on the dry matter content, the moisture contents, as previously reported (3), were computed on a sodium chloride-free basis for each side. When expressed as such, the mean moisture content was found to be 74.03% with a standard deviation of 0.70%, as compared with a mean of 71.38% and a standard deviation of 1.58% (3) calculated on the basis of the whole bacon. It is therefore evident that the direct effect of variations in the chloride content was a major source of variation in the moisture content of different sides.

The correlation between moisture, on a salt-free basis, and sodium chloride content was not significant ($r = 0.23$, for 40 degrees of freedom). The negative relation between the chloride and moisture contents of whole bacon, noted previously, was therefore due entirely to the additional dry matter contributed by the sodium chloride. These findings, together with the fact that the moisture content of bacon on a sodium chloride-free basis was essentially the same as that of fresh pork, indicate that the net result of pumping and curing is to increase the salt content, with little, if any, loss of moisture.

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